Figure 16

Method for Obtaining Non-Stochastically Generated Polypeptides that can induce a Broad-Spectrum Immune Response.

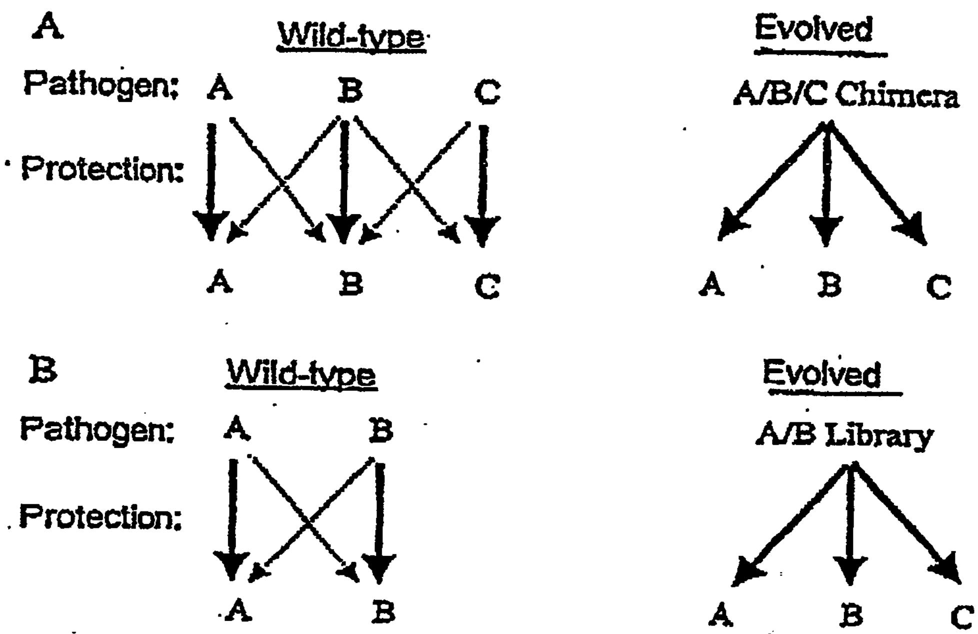


Figure 17

Possible factors for determining whether a particular polynucleotide encodes an immunogenic polypeptide having a desired property.

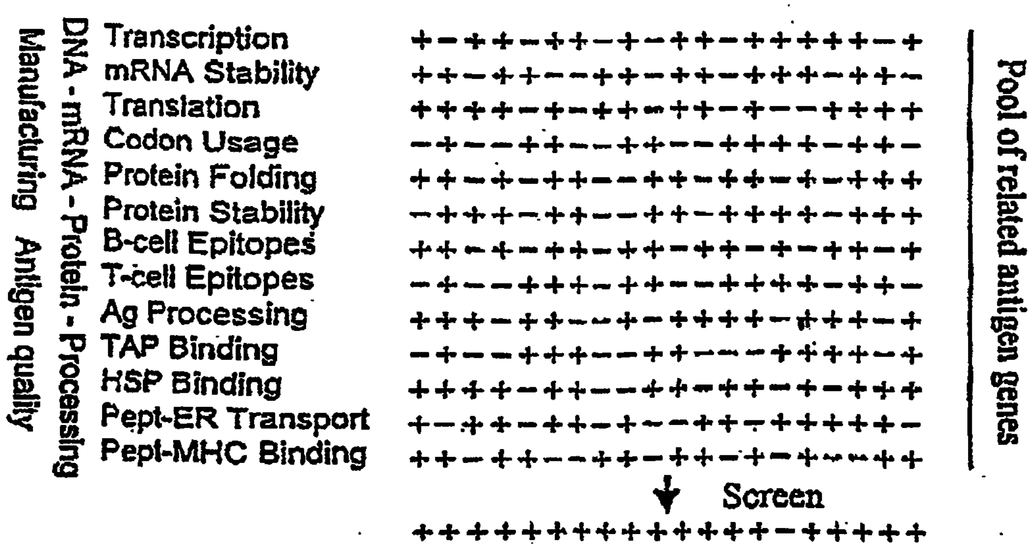


Figure 18

Screening strategy for antigen library screening.

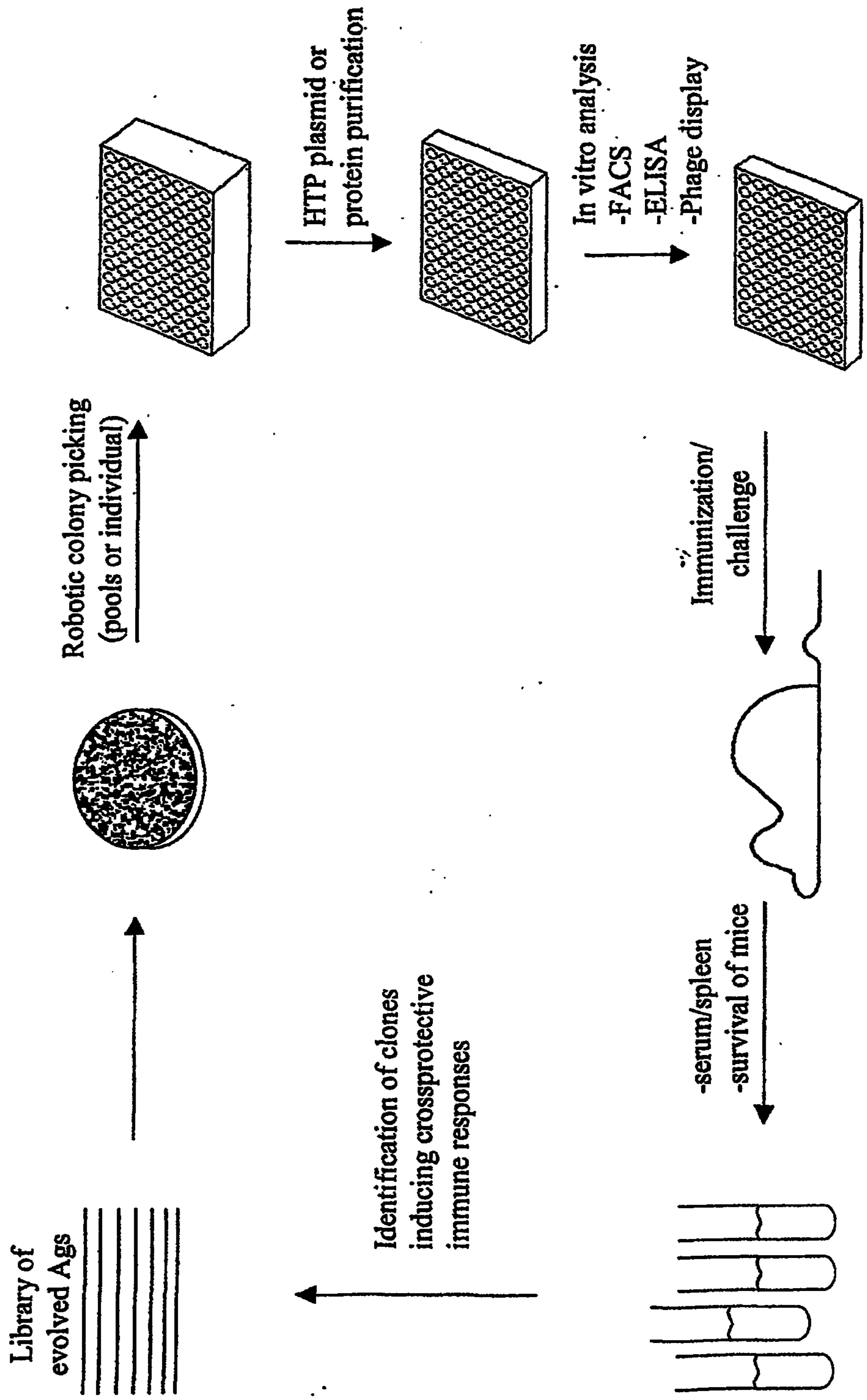


Figure 19

Strategy for pooling and deconvolution as used in antigen library screening

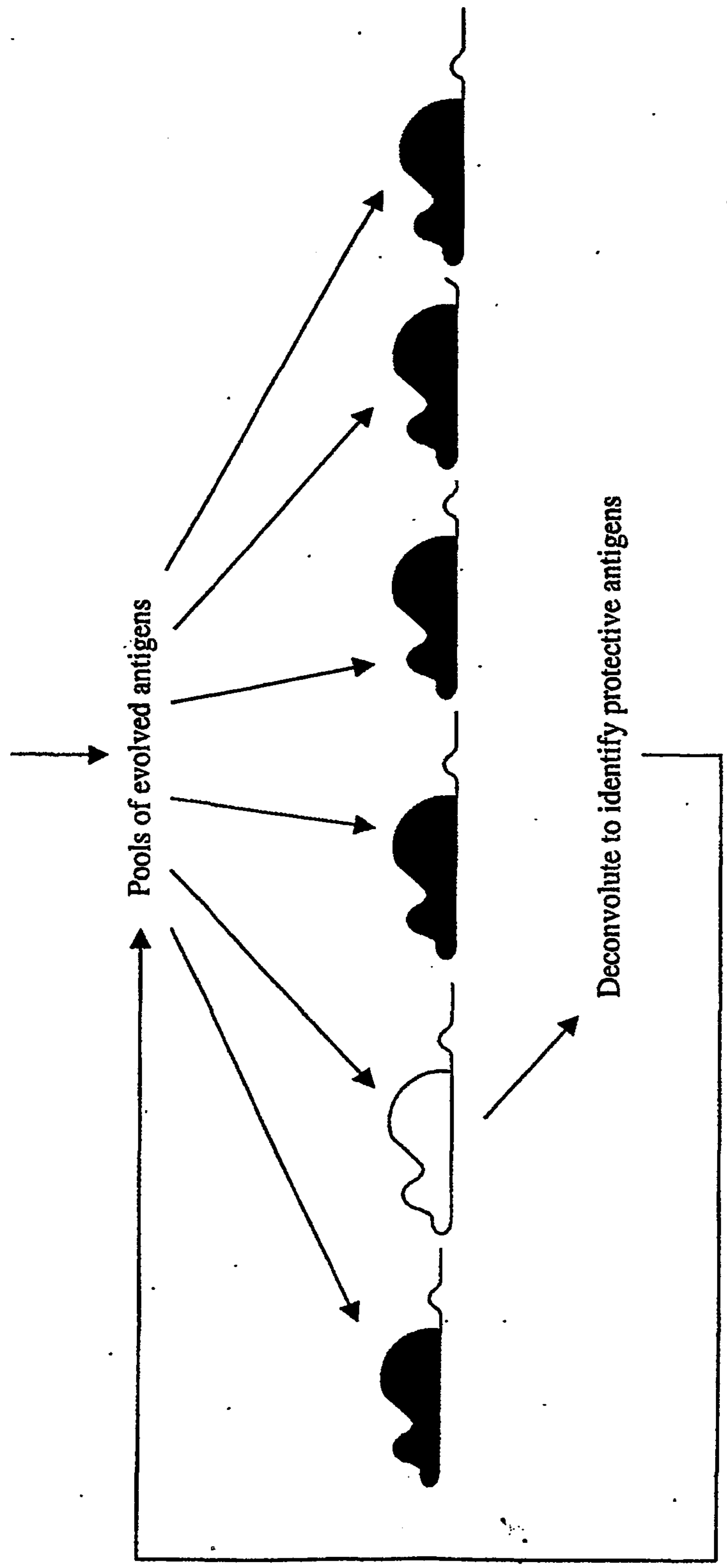
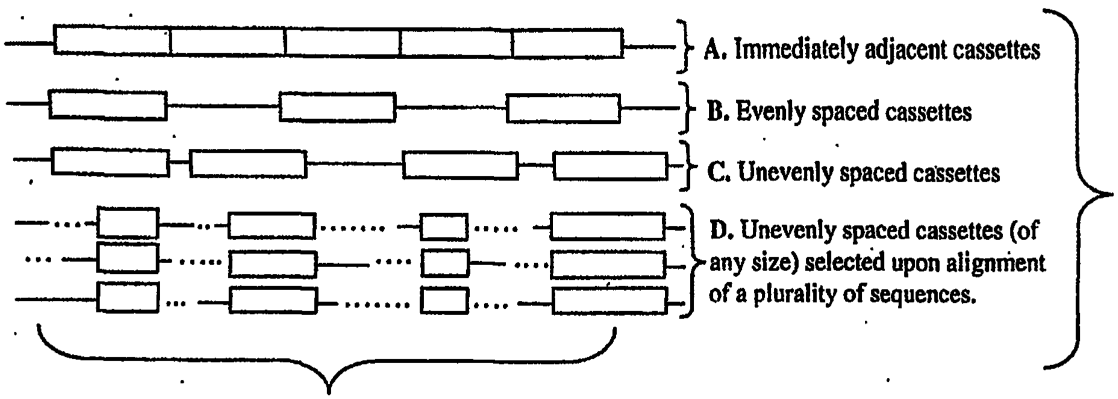


Figure 20. Preferred embodiments of site-saturation mutagenesis



I. SEQUENCES TO BE MUTAGENIZED

Nucleic acid sequences that can be mutagenized by non-stochastic site-saturation mutagenesis include any polynucleotide of 15-100,000 bases in length (e.g. any coding region & any non-coding region).

Specific examples:

- Promoter
- Enhancer
- Intron
- Whole gene
- cDNA
- Gene segment
- Operator
- Polynucleotide functional group
- Expression regulatory sequence
- Expressed sequence tag (EST)
- Open reading frame (ORF)
- Repressor/transactivator
- Origin of replication
- Gene pathway

II. MUTAGENIC CASSETTES WITHIN SEQUENCE TO BE MUTAGENIZED ($\boxed{}$)

Mutagenic cassettes that can be mutagenized by non-stochastic site-saturation mutagenesis include any polynucleotide cassette of 1-500 bases in length. Site-saturation mutagenesis is servicable for mutagenizing a complete set of cassettes contained within a polynucleotide sequence to be mutagenized. As shown, cassettes can be spaced along each polynucleotide differently (i.e. immediately adjacent, evenly spaced, or unevenly spaced) and of any size. In a preferred but non-limiting exemplification a set of mutagenic cassettes is a set of contiguous codons within a sequence of defined length. Alternatively, in another preferred but non-limiting example, a set of mutagenic cassettes is a set of nucleotide cassettes that are not shared by aligned related polynucleotides.

III. TYPES OF MUTATIONS THAT CAN BE INTRODUCED INTO MUTAGENIC CASSETTES

The type of mutations to be introduced in a set of mutagenic cassettes can be of the same type or of different types within each round of polynucleotide site-saturation mutagenesis. Each mutagenic cassette (within the nucleic acid sequence to be mutagenized) preferably is usually mutagenized by the use of a corresponding oligo (including by a degenerate oligo). Examples of degenerate mutations provided by this invention include:

- Codons for all 20 amino acids (e.g. N,N,N or N,N,G/T or N,N,G/C)
- All degenerate codons that do not change the amino acid sequence of the parental template (i.e. codons for the same amino acid that is present in the parental template)
- Codons (all or selected) for amino acids within the same grouping according to the selected amino acid grouping scheme*.
- Codons for at least 1 amino acid in each amino acids group*.

*Exemplary amino acid grouping schemes (notes, some groups overlap each other):

- **Aromatic** (Phe, Trp, Tyr)
- **Aliphatic** (Gly, Ala, Val, Leu, Ile)
- **OH-containing** (Ser, Tyr, Thr)
- **Acidic** (Asp, Glu, Asn, Gln)
- **Basic** (Lys, Arg, His)
- **Sulfur-containing** (Met, Cys)
- **Polar** (Ser, Thr, Cys, Asn, Gln, Tyr)
- **Non-polar** (Gly, Ala, Val, Leu, Ile, Met, Phe,Trp, Pro)

Figure 21

Schematic representation of a multimodule genetic vaccine vector (relative sizes of functional units are not drawn to scale)

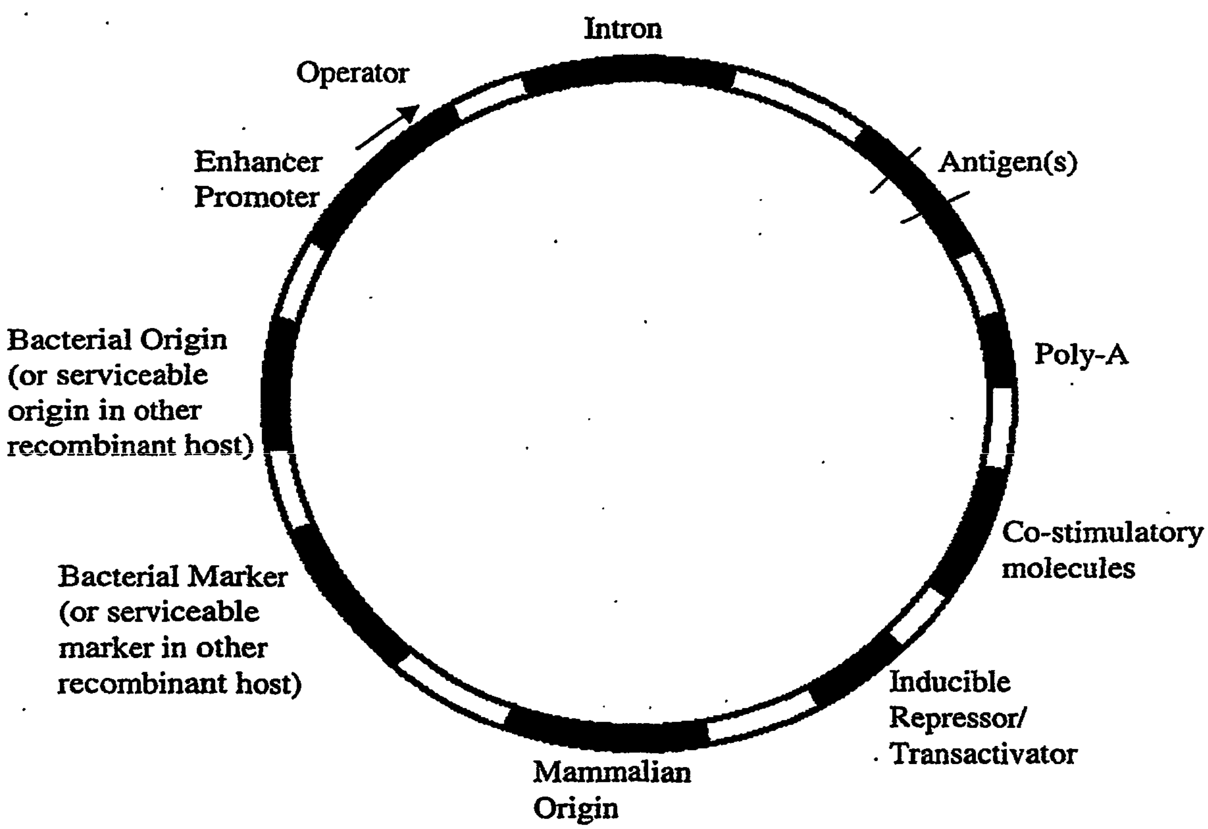


Figure 22A and 22B

Generation of vectors with multiple T cell epitopes.

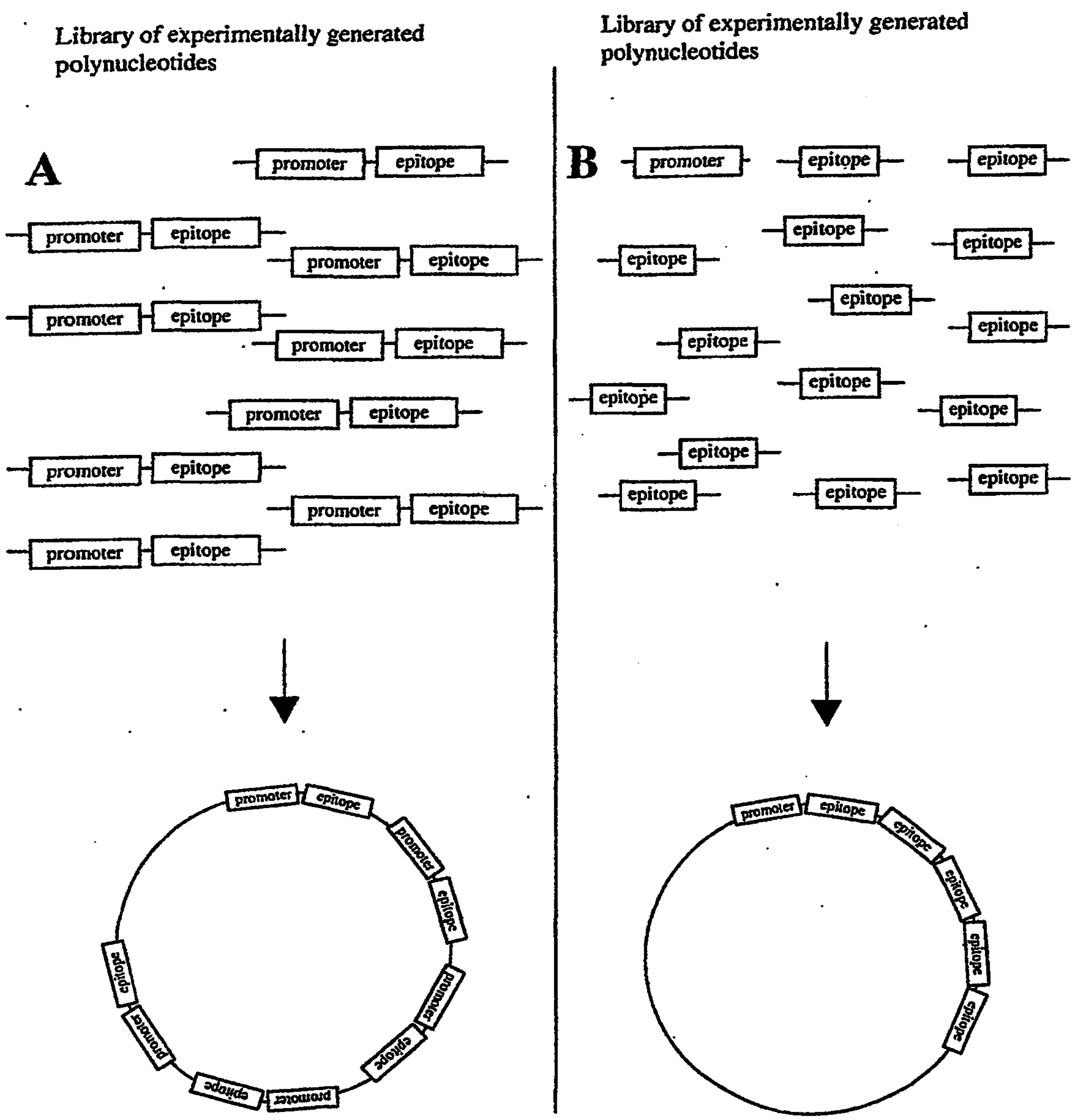


Figure 23

Generation of optimized genetic vaccines by directed evolution

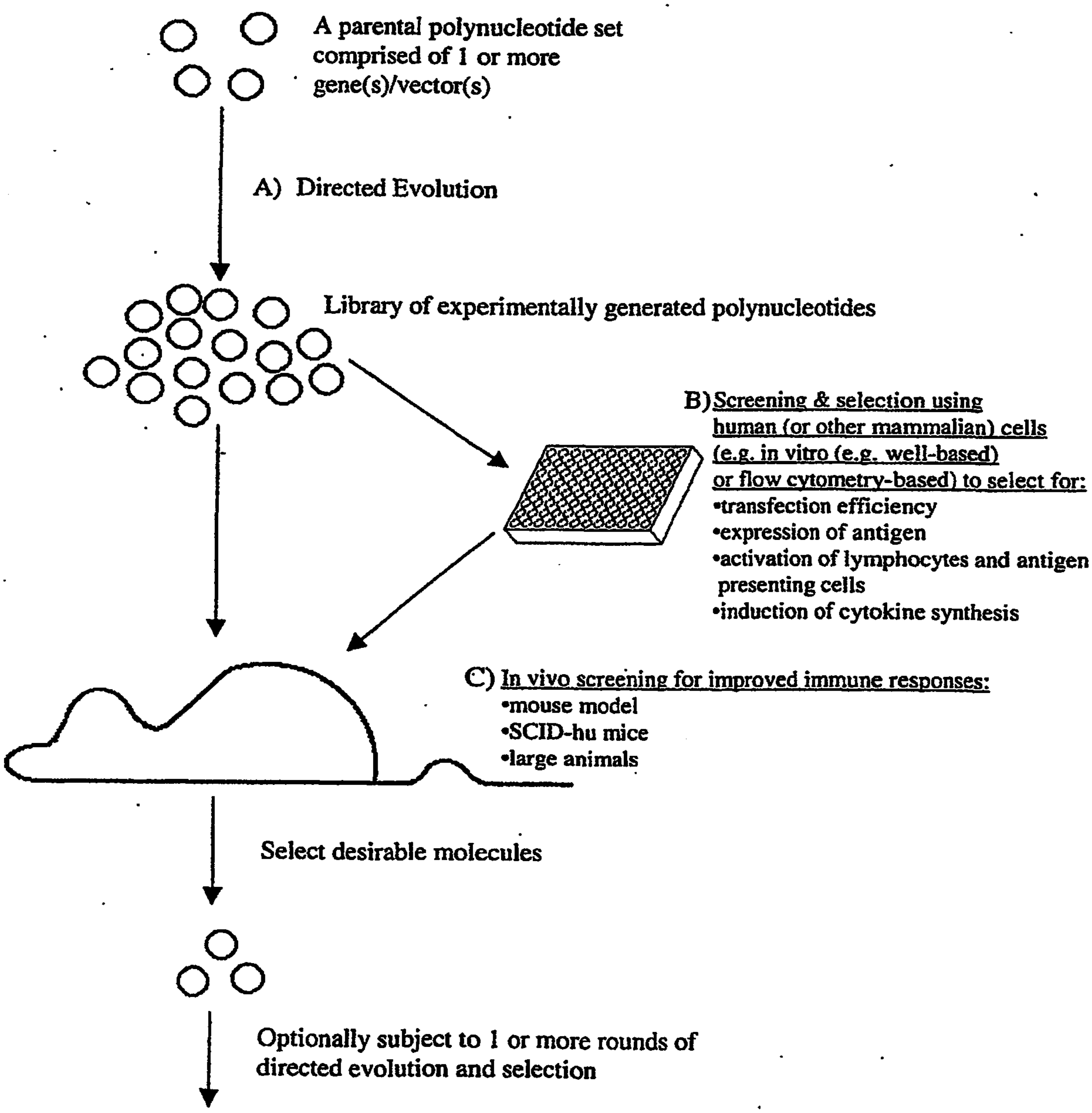


Figure 24

Recursive application of directed evolution and selection of evolved promoter sequences as an example of flow cytometry-based screening methods.

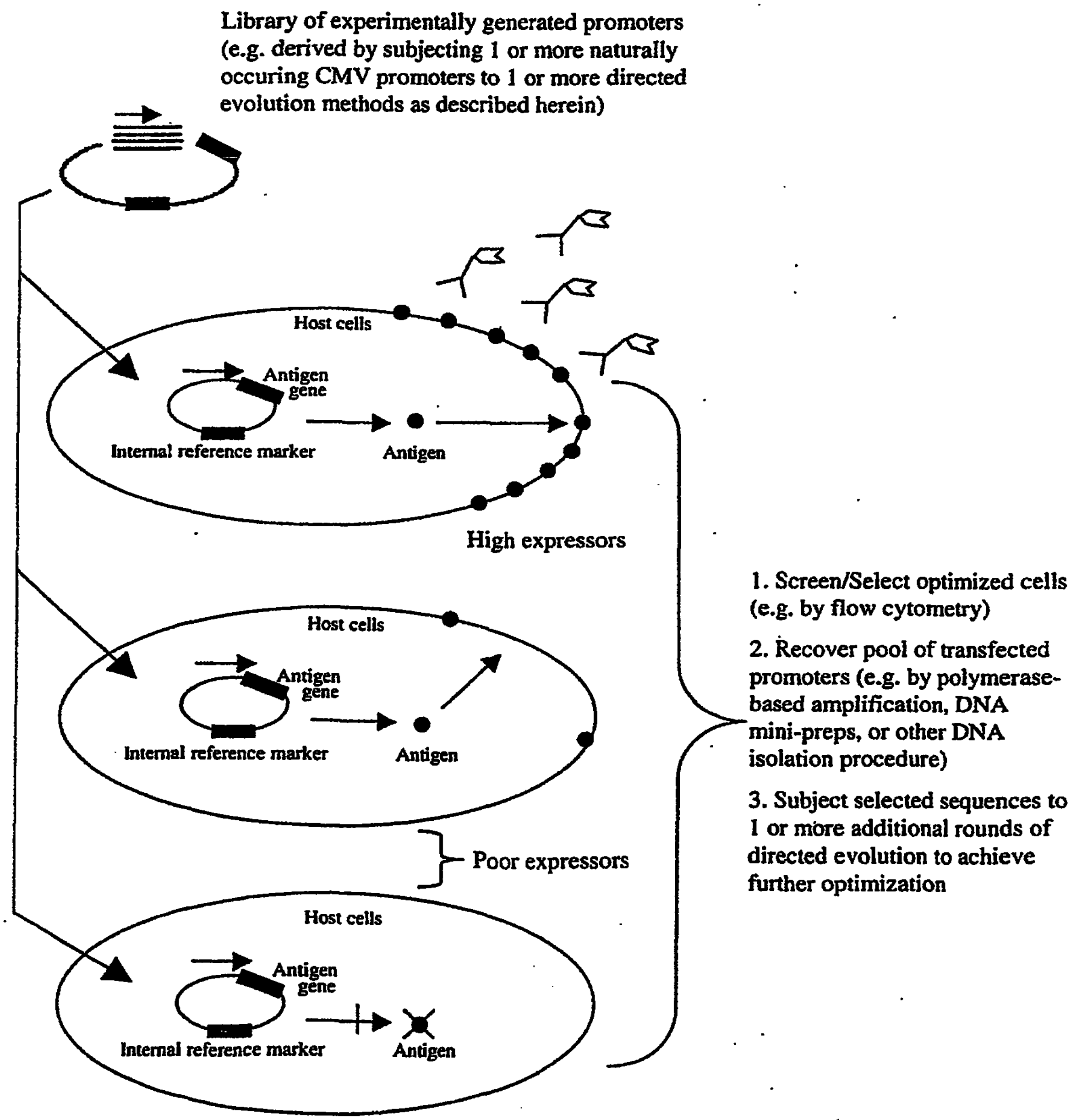


Figure 25

An apparatus for microinjections of skin and muscle.

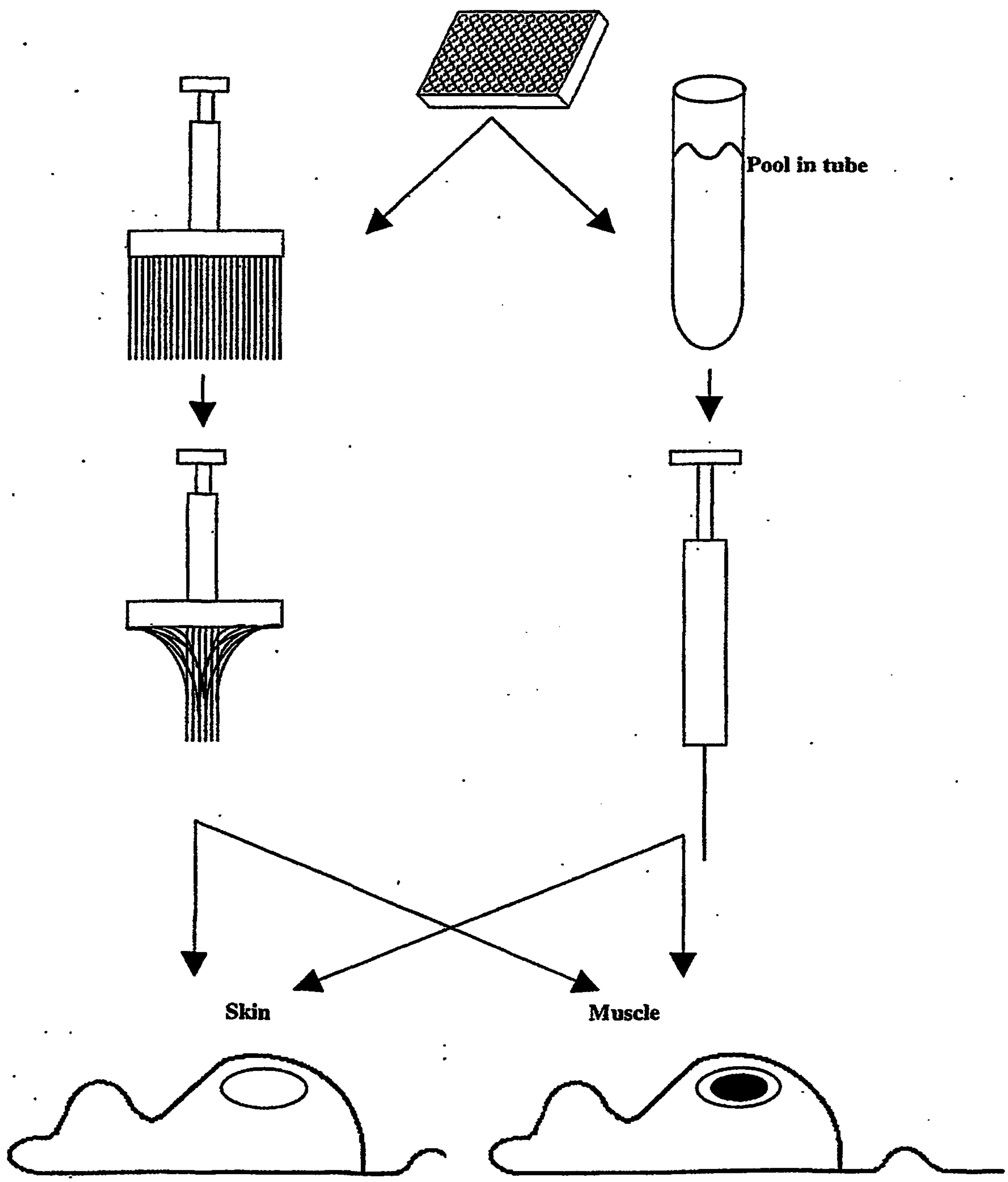


Figure 26 Panel A

Non-stochastic polynucleotide reassembly in combination with non-stochastic polynucleotide site-saturation mutagenesis.

Shown below is a non-limiting example of a permutation of the directed evolution methods described herein

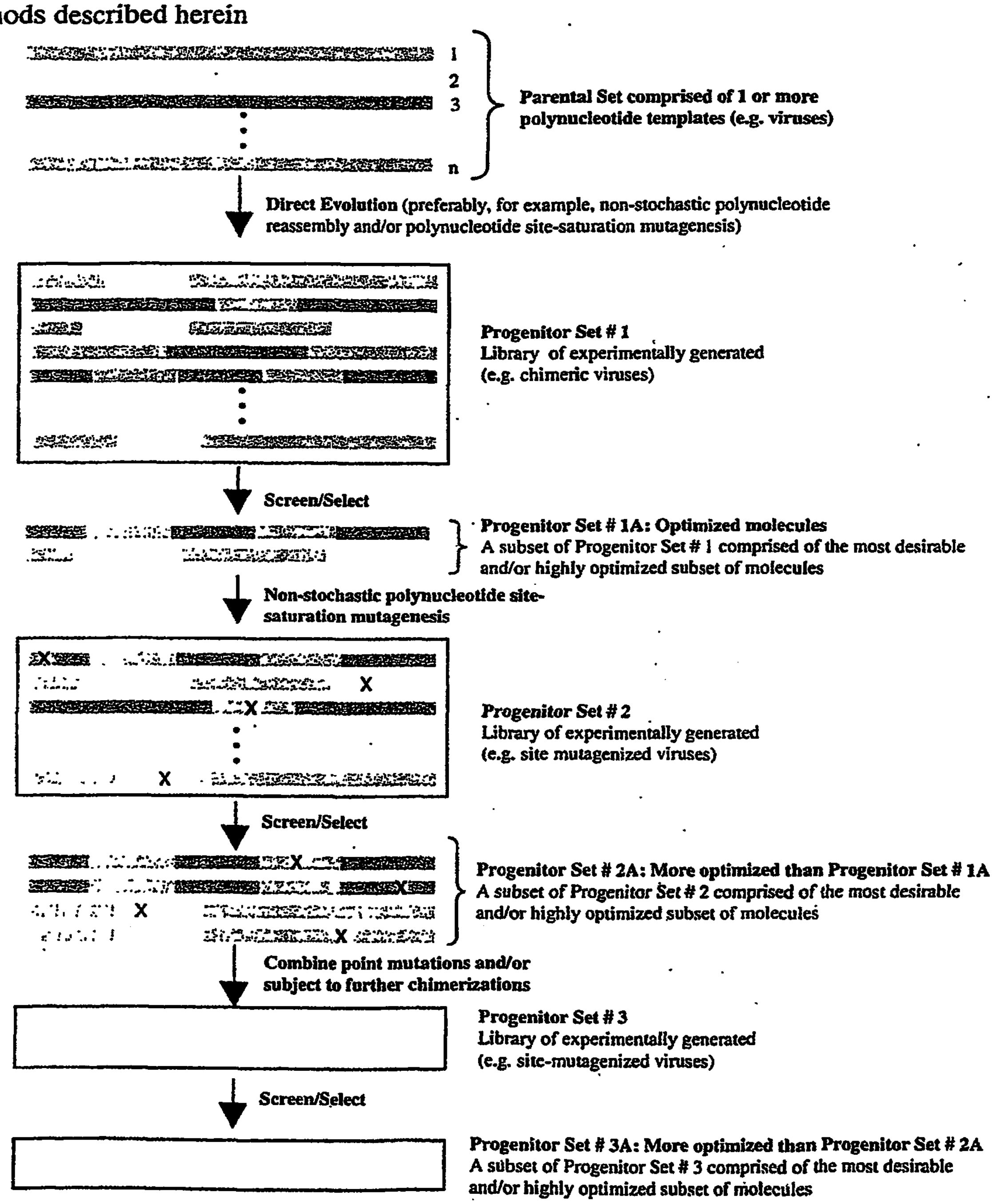


Figure 26 (continued) Panel B

Screening of experimentally generated molecules produced by non-stochastic polynucleotide reassembly in combination with non-stochastic polynucleotide site-saturation mutagenesis

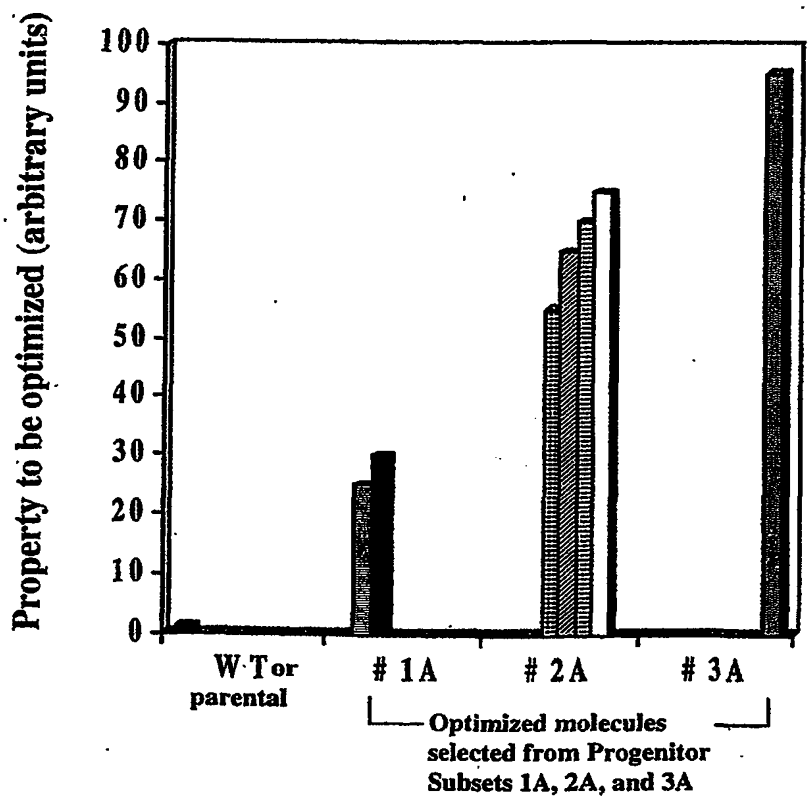


Figure 27

Vector for promoter evolution

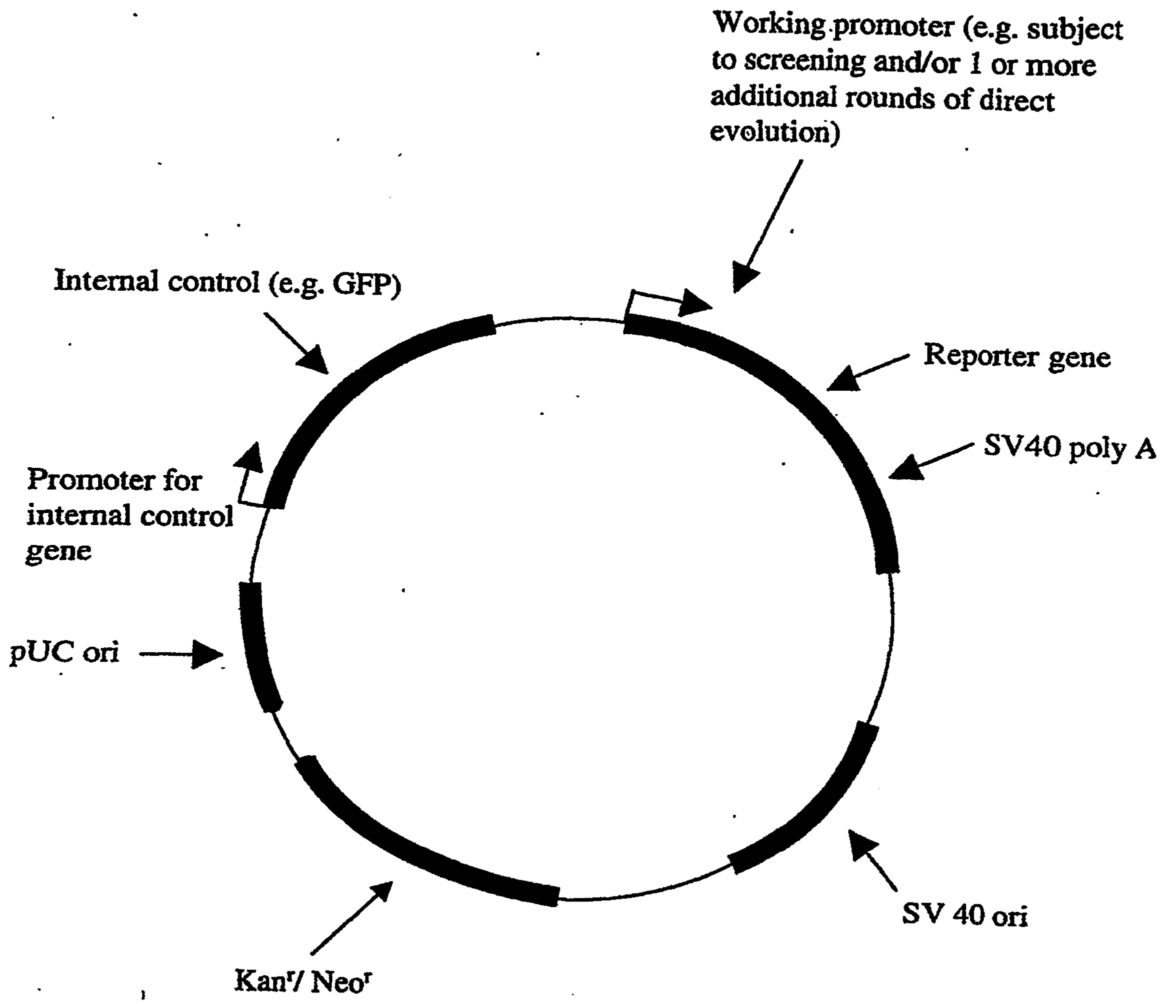


Figure 28

Iterative evolution of inducible promoters using directed evolution and flow cytometry-based selection.

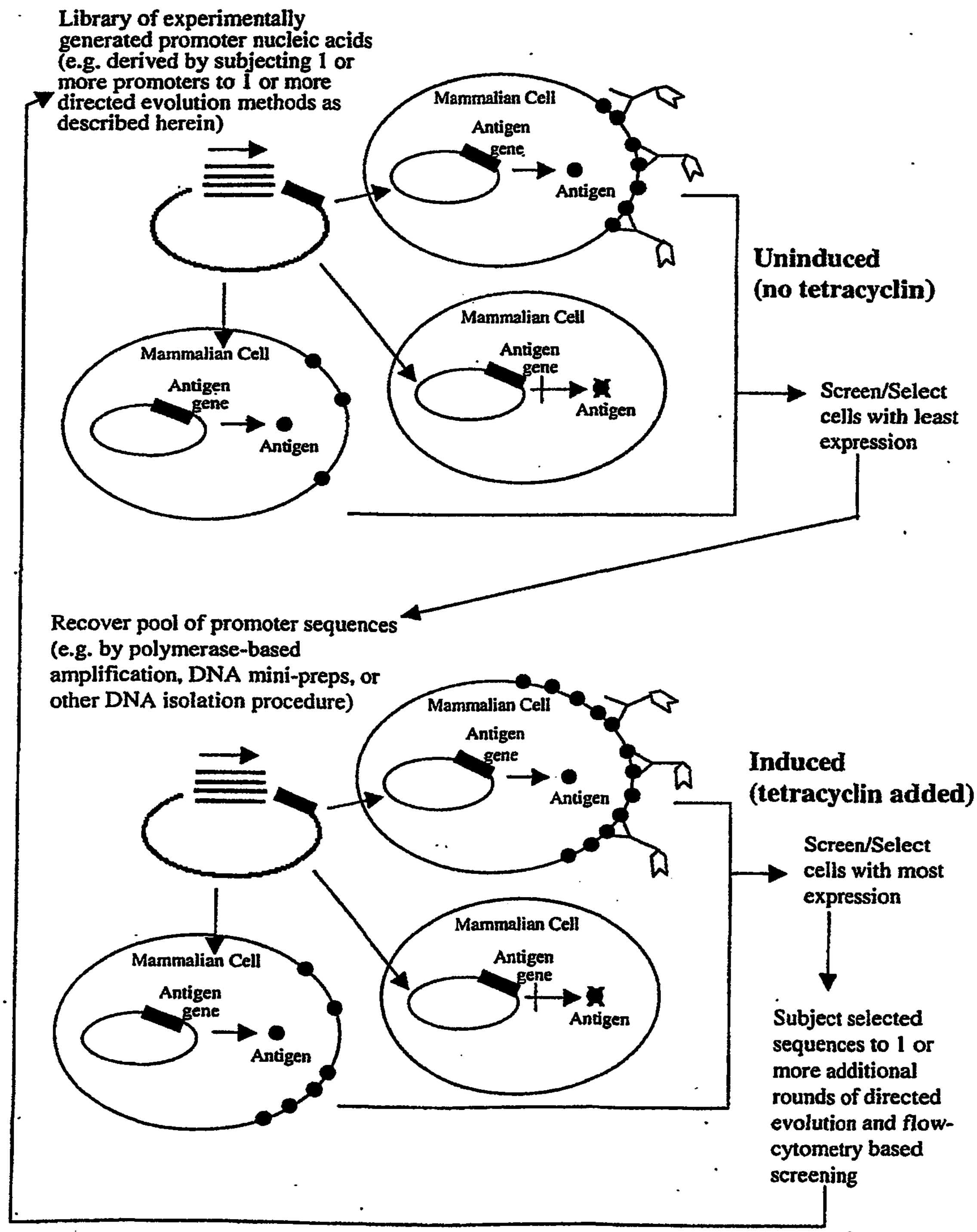


Figure 29

The present invention provides that a genetic vaccine can be subjected to directed evolution in order to achieve improved effectiveness upon administration by oral, intravenous, intramuscular, intradermal, anal, vaginal, or topical delivery methods.

The figure below shows an example of the directed evolution of a genetic vaccine, comprised of an M13 phage-based vaccine, to achieve optimization for oral delivery.

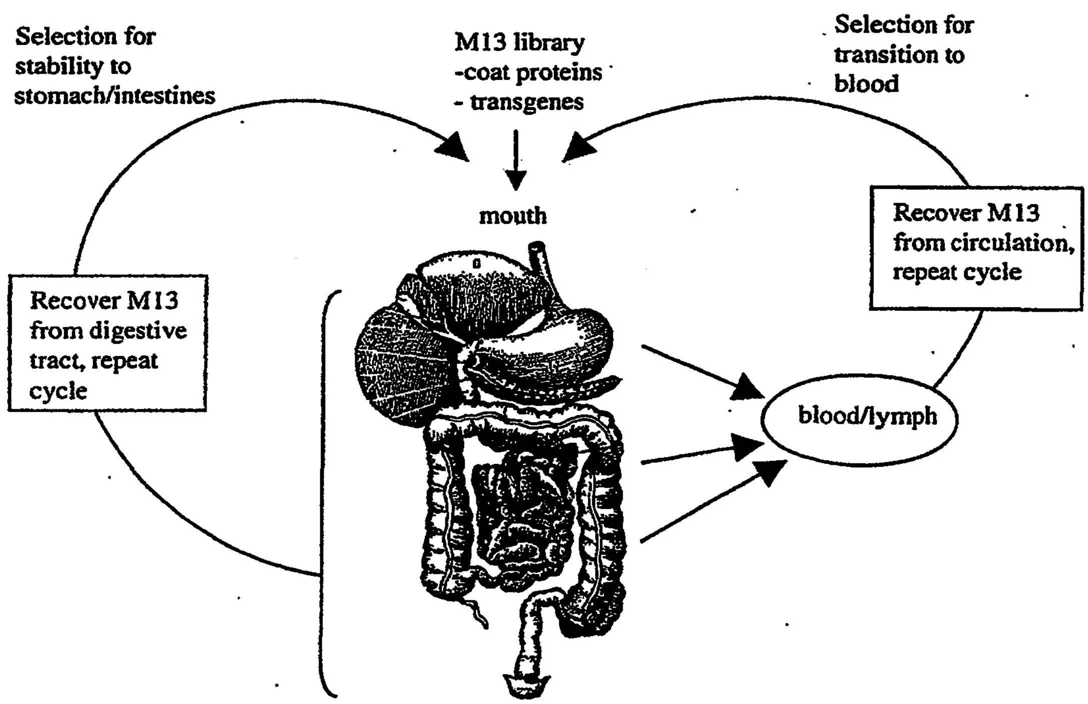


Figure 30

An alignment of the nucleotide sequences of two human CMV strains and one monkey strain.

		1 ... 50
AF026939 CMV	(1)	-----GTGGAAAGGTG-------TTCAGCATTT-GCTTGGAATCAGTAAG
AF047524 hum UL104	(1)	--GTAGTGAAATCCGTATGGACCTCCAGCACGCCGGCGGATGATCAGGGCC
AF078102 Rhesus	(1)	ATGGATTTAAA-GTGGCCGA---TTGAGGTTGTCGCTGAAGACTTTGTGA
		51 ... 100
AF026939 CMV	(38)	CTAAAAACAAAATCAACCGGGAGCCCAGCTTTTCAGAACTGGAGGGAAA-
AF047524 hum UL104	(49)	TCGATTTCGAAATGGGG--CGACAC--GCTCTGG-GCCGCGCCGCTCCTC
AF078102 Rhesus	(47)	GTGTTTTCGAAGGTAGATATGACAGT-GCTTTCCAGAAGTGCCCCTGAA-
		101 ... 150
AF026939 CMV	(87)	--CAGCCATCATGAGTGAGGTCACCAAGAATTCCCTGGAGAAAATCCT--
AF047524 hum UL104	(94)	GTCTGCCGTGATCAGGCGGCGGCGGCCG--GACCTTTGAAGCGTTCCTGG
AF078102 Rhesus	(95)	GCCTGGGGTACATATCTGAAGCCACCGAGATGAGCGTCC-GAGGACGTGA
		151 ... 200
AF026939 CMV	(133)	CCGACAGGTGAAATGCCGATTTGAG---CTGGAACTATTCAAGGAAGACA
AF047524 hum UL104	(142)	GCCCCCGCT-CGAGGCAGTTCCCCTTTCTGGCAGTCCGGCGCCGCTTCG
AF078102 Rhesus	(144)	TACGGTGGGACTACGGCGTCTTCAGTTTGCGTAATCT-CGTGTTCCAACG
		201 ... 250
AF026939 CMV	(180)	GTGTCTCA--AGGGATCTAGAAGATAGAGTGTG--T---AACCAGATTGA
AF047524 hum UL104	(191)	CGG-CTCATTTGGCGTCGGCGCGCCTTCTCGCGGCTGCAAATCAGCTCCA
AF078102 Rhesus	(193)	TTGTCTGACTATGTTACAGGGTGTTTATCTGCCAC----AATACGATCAA
		251 ... 300
AF026939 CMV	(223)	A----TTTTTAAACACTGAGTTCAAAGCTACAATGTAGAACTTGTTGGCC
AF047524 hum UL104	(240)	C-GTATCGGCAAAAACTTGCTGTCGTCGTAGGCGGCGGCACGATCTCGCC
AF078102 Rhesus	(239)	CCGTGTTTAGGAGAATATATTAAAAAGAATTGTGGTACTATCATTCCGTT
		301 ... 350
AF026939 CMV	(269)	TACATAAAAGACGTAGATGGTAAGAACGAGGCAGCCCTGGAAT-GCTTAC
AF047524 hum UL104	(289)	GAAGGAGAGCTGCAGGTAGGCCTCGGGTACGG-GGTCCAGCGT-GCCCAG
AF078102 Rhesus	(289)	GCGCAACCCGTGTAGCT-GGTTTCTCGTCCTTCGCTCTGGTATAGATATT
		351 ... 400
AF026939 CMV	(318)	GGGAAGCTGAAGAGTTA----ATGCAGCAAGAACATGCTGACCAAGCAGA
AF047524 hum UL104	(337)	CGGCAGGATGTGACACA---GATAGGGGAGGGTCACGGGCTCTACCGTGT
AF078102 Rhesus	(338)	GCCGAGGTAGAAACCCGTTCGTTGGACGAGTATCTTT-GCTGCAAAGAGA
		401 ... 450
AF026939 CMV	(364)	AATCAGA--AGTGTA--GTGACTTGGGGAAACT-ACGGCTGG-GTCTACT
AF047524 hum UL104	(384)	AATTGGAGTAGACGATGGCCTGTTCGGCCCCGTGATGGGTGA--CGAGAC
AF078102 Rhesus	(387)	AATTGGA--AGCACTGGCAATATTGGGGGTGCAGTTGGGTGATGGCAGTG
		451 ... 500
AF026939 CMV	(408)	ATGAGTTGGGCA----GACTCTCAGATGCTCAGATTT---ATGTAGATAA
AF047524 hum UL104	(432)	GCGGCAGGCGAA---AGGTGGGGAATAGTCGTTTTTCCCACAGCTGCG-T
AF078102 Rhesus	(435)	ATGGCAAAAAAATCCAGGAGACTACGTGTGTGGTTCTGCTGGGAAGCTAT
		501 ... 550
AF026939 CMV	(451)	GGTGAAACAAAGCTGCAAGAAATTTTCAAATCGATACAGTATTGAGTATT
AF047524 hum UL104	(478)	GAGGAAGCGTTGCAGCGACTGGGTGCGGGCACGAACTGC-GAGAAGAAG
AF078102 Rhesus	(485)	GGATTTGTGTACGTGTATGAGTGGGACAGTGACGGACTCT-TTCAGATAG

Figure 30 continued

```
                              551                                                600
       AF026939 CMV   (501) CTGAACTTGACTGTGAGGAAGGGTGGACACAACTGAAGTGTGGAAGAAAT
 AF047524 hum UL104   (527) CAG--TTGGCGAGCAGG--CGGTTGTCGTGCAGCGCC-AACGGACG-GAA
    AF078102 Rhesus   (534) GTA--GCTCAGTCAAAG--AACTCGCGCAGCAGGGTT-TACTGACATGTG

                              601                                                650
       AF026939 CMV   (551) GAAAGGGCGAAGGTGTGTTTTGAGAAGGGTGT---GGAAGAA-AAGCCCA
 AF047524 hum UL104   (571) AGGGGCCGCGTCGCGCGGCTTGCGCAGGGCGG---GCAACAC-GGGG--A
    AF078102 Rhesus   (579) AATCGGTATATCGCCATCG--GCAAACGCCCTTCTCCACCAGCGAGCCTC

                              651                                                700
       AF026939 CMV   (597) ACAACGCAGAATTCTCC-TCTGGACTGGGAATTGCGATGTACCATCTGGA
 AF047524 hum UL104   (615) GGTG----GTAGAG----TTCGGCGTGGCGGCGGCCCAGGCTCATGGAGT
    AF078102 Rhesus   (627) GTTTTCAAGTCGAGAAATTGTTGTGTGTGGATCCGACAGACGCACGTGGT

                              701                                                750
       AF026939 CMV   (646) T-AATGACGCAGAGAAACAGTTCTCTACTGATGTTTTGAAG------CAG
 AF047524 hum UL104   (657) GCTCGGGGCGGCGAGGCGTAGCGGGTGA-GCAGGTCGGCAGTTCGCGCAC
    AF078102 Rhesus   (677) G-TAGGTAAGACCGCAGAAGAGTTTCATGGCGTTAACGTAGTA-GTGAAA

                              751                                                800
       AF026939 CMV   (689) GCGATTGAGGTGAGTGCTGATAACGAAT---ACGTGAAGGTTGTCTTGGG
 AF047524 hum UL104   (706) GCGATTCTCGCAGGTGTGGTTGAGGGTGCGCAGGTCCTGGATCTCGTCTA
    AF078102 Rhesus   (725) ACGCCTGGGGGATCGGAGGCCGATGCCTTGCTGCTTCTGGGTACTGTGGA

                              801                                                850
       AF026939 CMV   (736) CCTGAAACTGCAGAAGATGAATAAAGA--AGCTGAAGGAGAGCAGTTTGT
 AF047524 hum UL104   (756) GCTGGGACTGGATCTGCTCGTGCAGGG--ACTTGAT---GACCTGCTTCT
    AF078102 Rhesus   (775) GAAGCTAGGTCAAATGTATCCGTTTGCTAAAATGA----GAGCTGATAAC

                              851                                                900
       AF026939 CMV   (784) TGAAGAAGCCTTGGAAAAGTCTCCTTGCCAAACAGATGTCCTCCGCAGTG
 AF047524 hum UL104   (801) TAAACAGGCTGGCGGATG-TCCGGCTCGGGGCGCCGGCGGCG--GGTG-G
    AF078102 Rhesus   (821) TTTAGTAATCTGATAAA-GTATATTAAT-CAACGCATGT-----GTTG-C

                              901                                                950
       AF026939 CMV   (834) GAGGCAAATTTTAGAGAAGAAAAGGTGACCTAGACAAAGCTATTGAACTG
 AF047524 hum UL104   (847) GGCCGGCAGCAGGCCGACGTGGCCCGGGGGTCGTGCCA-GCACGGCACCG
    AF078102 Rhesus   (863) GGATGGTACGTACTTGGTGTTACTGGCCGTTACGGTAA-TTTTGGAATTT

                              951                                               1000
       AF026939 CMV   (884) TTTCAACGGGTGTTGGAATCGACACGAAACAATGGCTACCTCTATCACCA
 AF047524 hum UL104   (896) CCGGGCCGC----ACCACGGCGGGTGCACCCGGACCAGGCGCGGGTAGCA
    AF078102 Rhesus   (912) TGCTGACCTGTGGAATAATTGTGTTGGACGAGTGCGGCGTGTGT-TATGT

                              1001                                              1050
       AF026939 CMV   (934) GATTGGGTGCTGGTACAAGGCAAAAGTAAGACAAATGCAGAATACAGGAG
 AF047524 hum UL104   (942) GACGGTTTTGGTCCACCAGCGAAGGGGTCAAGTCCTGCAGG------AAG
    AF078102 Rhesus   (961) GCTGAGAATCGACGAA-AGCGACGTGTTTC-GTCTAGCCGAT-----AAC

                              1051                                              1100
       AF026939 CMV   (984) AATCTGAAGCTAGTGGAAATAAAGAGATG--ATTGAAGCACTAAAGCAAT
 AF047524 hum UL104   (986) GACTCGACGCTGTCCTCGATGGCGATGCGCGATTTGCTGTCCGAGACGTT
    AF078102 Rhesus  (1004) ATCGAAATGTTGTTCA-GATGCGGCTTTC----TTAAGCTCCGTGGCATT

                              1101                                              1150
       AF026939 CMV  (1032) ATGGTATGGACTATTCGAATAAAGCTCTTGAGAAGGGACTGAATCCTCTG
 AF047524 hum UL104  (1036) AAGCAAAAACTTCATAATGGACTTTT-TGGCGTCGCT-GCCCCGGTCGTG
    AF078102 Rhesus  (1049) ACGCGAT---TTGACCGCGGATTGCG-TGGCGAGGCTCGTCTGGAGAGTG
```

Figure 30 continued

```
                        1151                                              1200
AF026939 CMV     (1082) AATGGATACTCCGATCTGGCTGAG-TTGCTGGAGACGGAATGTTATCAGA
AF047524 hum UL104 (1084) C-TGG-TCCATG-ATGTGCACCAGCTTCTTGCAGTTGAGCTCGTGGCGGC
AF078102 Rhesus  (1095) AGTGG-TAGTGGTATGAGGGTGCCCGTAAGCAAGATCT-GTCTTGGTGCT

                        1201                                              1250
AF026939 CMV     (1131) GACGATTGAATTAAGGAAGTGCGTGAT--GCTGAAAAGGGAAGAATG----G
AF047524 hum UL104 (1131) TGGCGGTGACCGAG---TTGCAGAGGAAAGGTATTGAGGAACTGGGAGATC
AF078102 Rhesus  (1143) GTATGGTGAACAG---AGTTAGCGCC--------GAGGAGGCTC-------

                        1251                                              1300
AF026939 CMV     (1175) CATCAGCG-GTAGTGCAAGGTTGA--GAAATATAATGGGAAGTCTGAAGA
AF047524 hum UL104 (1178) TTTTGGTGCGGGCAGAGCCGCGTGGTAGGGCAG-AATGTG-CTCGTGCAGG
AF078102 Rhesus  (1175) -TGTGGTG-GGTACGACTGGCTGAC-GCGCAGTAATCGGGCTCATGATAA

                        1301                                              1350
AF026939 CMV     (1222) CACTGGTGTGGAAGATGGTTTAGAGG-GTTTGTC-----CATAAGCAAAA
AF047524 hum UL104 (1226) TGTGCGAC--GGGGGTGGTGAACAGCAGCTTGTGGCGCTCATAAGCCAGC
AF078102 Rhesus  (1222) AATAGGTA--GCAGTTGTTGTTGGGGACGTAGT---GATTTGATGCCTAC

                        1351                                              1400
AF026939 CMV     (1266) AATCAACTGAGAAG-GAAGAGATCAAAGACGAACGACAGAATGTATCCGA
AF047524 hum UL104 (1274) GGTTCGGTCGGCAC-GTACAAGCGGATGTGGTTGGCGCGCAGCTGCGCCT
AF078102 Rhesus  (1267) CGGAGTATTGCAAGAGAATCAGAATTGGTGGTTTCCGTGGAGCAGTGTCA

                        1401                                              1450
AF026939 CMV     (1315) AAATCTGGTTGCACAAAATGCACCAAATTATTG----GTATCTTCAAGGA
AF047524 hum UL104 (1323) GCAGCCGCTCG----GAGCGCACCTTCTTGAAGACGCGTACCT--CGGGC
AF078102 Rhesus  (1317) C--GGTTGTAG----AAGGGGCCCAAAGCGGTG---CGTGGGA--AGAAA

                        1451                                              1500
AF026939 CMV     (1361) TTAATTCATAAGGAGAATGGAGATGTGGTGGAAGCAGCGAAATGTTATGA
AF047524 hum UL104 (1367) GCGTTGGCTACGCGCA---CAGCTGCCAGGGGCTCGGCCTCCTGCAGCA-
AF078102 Rhesus  (1356) ATGATGAGTTAAGGGA---GAAAACGTGTGATGGTACTGTGATGTTTCGT

                        1501                                              1550
AF026939 CMV     (1411) GAAGGAAGTGGGCCGCCTGG--TAAGGGATGGCGGTTGAGGCAT--AGGC
AF047524 hum UL104 (1413) GCAGCGCCCAGGTTAGCCTGGAGCAGGTGCTGGCGCCAGCGGGTGTGTCT-C
AF078102 Rhesus  (1403) GTAGACAGAGGTTTTTCTTCG--GAGAAGGGTAAAGTTGGAGGGTGAAAGC

                        1551                                              1600
AF026939 CMV     (1457) AGTATTTTGCTGTCAGCATGTGAGGTTGAGGATGGT-AGTGAGGAAATGG
AF047524 hum UL104 (1462) GGTGGCT-GGTGCAC-GGGCGCGGGTACAAATTGC--GCCGCTCGGCC
AF078102 Rhesus  (1451) GATGAGTACAGAGAAGAGGAGGAAGATGATGATAAGCAGTCAGAAGAAAC

                        1601                                              1650
AF026939 CMV     (1506) GGGAGGGG-GGAGTCAGCTGCA---GTGCCAGAGAGCTCCGTCTGTAACTC
AF047524 hum UL104 (1508) GCGTG-GG-TGGGCTTGGTGGTTCACGTGCAGCAGCGGTAC---CAGTCCC
AF078102 Rhesus  (1501) CGGTGAGGATGAGCCTGTTGTCATTGGTTCACAT-GAAAC---AAACGGC

                        1651                                              1700
AF026939 CMV     (1552) AGAGCAACTGAACTGAGACAGAGGAGGAAACAGAGC--ATCAGAAGCCT
AF047524 hum UL104 (1553) ACCGTTAC-GCACCAATCGACGTAGAGACCATAGTCGTCGTTATGGGCGT
AF078102 Rhesus  (1547) AGTGTTG--------ATTGTTATGATGATGAGAGTGA--GTCATCTCTGT

                        1701                                              1750
AF026939 CMV     (1600) GGAGTGGTGGTTGTGACGGGTAGGAGGATAGGAAGAGAG-GGGGCGCCAA
AF047524 hum UL104 (1602) ACTGATATAAA-ATGTCGCG---GAGCGCGGGAG-G---ACGCCCGTTT
AF078102 Rhesus  (1587) CGTCAGATACCCATGATCG-----ACCAGCCCCAGACATTATGGCCATAA
```

Figure 31

An alignment of IL-4 nucleotide sequences from 3 species (human, primate, and canine).

```
                                       1                                                50
         AF187322 Canis IL-4     (1)  TGCATCGTTAGCGTCTCCTAGTAAACTGATTTGTCTGCTATTGTCACTGC
    NM_000589 Homo sapien IL-4     (1)  TGCATCGTTAGCTTCTCCTGATAAACTAATT-GCCTCACATTGTCACTGC
       U19838 Cercocebus IL-4     (1)  --------------------------------------------------

                                       51                                              100
         AF187322 Canis IL-4    (51)  AAATAGAGATCTATTAATGGGTCTCACCTCCCAACTGATTCCAACTCTGG
    NM_000589 Homo sapien IL-4    (50)  AAATCGACACCTATTAATGGGTCTCACCTCCCAACTGCTTCCCCCTCTGT
       U19838 Cercocebus IL-4     (1)  ----------------ATGGGTCTCACCTCCCAACTGCTTCCCCCTCTGT

                                       101                                             150
         AF187322 Canis IL-4   (101)  TCTGCTTACTAGGACTCACCAGCACCTTTGTCCACGGACATAACTTCAAT
    NM_000589 Homo sapien IL-4   (100)  TCTTCCTGCTAGCATGTGCCGGCAACTTTGTCCACGGACACAAGTGCGAT
       U19838 Cercocebus IL-4    (35)  TCTTCCTGCTAGCATGTGCCGGCAACTTTGCCCATGGACACAACTGCCAT

                                       151                                             200
         AF187322 Canis IL-4   (151)  ATTACTATTAAAGAGATCATCAAAATCTTGAACATCCTCACAGCGAGAAA
    NM_000589 Homo sapien IL-4   (150)  ATCACCTTACAGGAGATCATCAAAACTTTGAACAGCCTCACAGAGC-AGA
       U19838 Cercocebus IL-4    (85)  ATCGCCTTACGGGAGATCATCGAAACTCTGAACAGCCTCACAGAGC-AGA

                                       201                                             250
         AF187322 Canis IL-4   (201)  CGACTC-GTCCATGGAGCTGACTGTCAAGGACGTCTTCACTGCTCCAAAG
    NM_000589 Homo sapien IL-4   (199)  AGACTCTGTGCACCGAGTTGACCGTAACAGACATCTTTGCTGCCTCCAAG
       U19838 Cercocebus IL-4   (134)  AGACTCTGTGCACCAAGTTGACGATAACGGACATCCTTGCTGCCTCCAAG

                                       251                                             300
         AF187322 Canis IL-4   (250)  AACACAAGCGATAAGGAAATCTTCTGCAGAGCTGCTACTGTACTGCGGCA
    NM_000589 Homo sapien IL-4   (249)  AACACAACTGAGAAGGAAACCTTCTGCAGGGCTGCGACTGTGCTCCGGCA
       U19838 Cercocebus IL-4   (184)  AACACAACTGAGAAGGAAACCTTCTGCAGGGCTGCGACTGTGCTCCGGCA

                                       301                                             350
         AF187322 Canis IL-4   (300)  GATCTATACACACAA---------------CTGG----------------
    NM_000589 Homo sapien IL-4   (299)  GTTCTACAGCCACCATGAGAAGGACACTCGCTGCCTGGGTGCGACTGCAC
       U19838 Cercocebus IL-4   (234)  GTTCTACAGCCACCATGAGAAGGACACTCGCTGCCTGGGTGCGAGTGCAC

                                       351                                             400
         AF187322 Canis IL-4   (319)  ------TCCA-----ACA------------GATATCTCAGAGGACTCTAC
    NM_000589 Homo sapien IL-4   (349)  AGCAGTTCCACAGGCACAAGGAGCTGATCCGATTCCTGAAACGGCTCGAC
       U19838 Cercocebus IL-4   (284)  AGCAGTTCCACAGGCACAAGCAGCTGATCCGATTCGTGAAACGGCTCGAC

                                       401                                             450
         AF187322 Canis IL-4   (346)  AGGAACCTCAGCAGGATGGCAAACAAGA---CCTGTTCTATGAATGAAAT
    NM_000589 Homo sapien IL-4   (399)  AGGAACCTCTGGGGCCTGGCGGGCTTGAATTCCTGTCCTGTGAAGGAAGC
       U19838 Cercocebus IL-4   (334)  AGGAACCTCTGGGGCCTGGCGGGCTTGAACTCCTGTCCTGTGAAGGAAGC

                                       451                                             500
         AF187322 Canis IL-4   (393)  CAAGAAGAGTACACTGAAAGACTTCTTGGAAAGGCTAAAAGTGATCATGC
    NM_000589 Homo sapien IL-4   (449)  CAACCAGAGTACGTTGGAAAACTTCTTGGAAAGGCTAAAGACGATCATGA
       U19838 Cercocebus IL-4   (384)  CAGCCAGAGTACGTTGGAAGACTTCTTGGAAAGGCTAAAGACGATCATGA

                                       501                                             550
         AF187322 Canis IL-4   (443)  AGAAGAAATACTACAGGCATTGAAGCTGAATATTTTAATTTATGAGTTTT
    NM_000589 Homo sapien IL-4   (499)  GAGAGAAATATTCAAAGTGTTCGAGCTGAATATTTTAATTTATGAGTTTT
       U19838 Cercocebus IL-4   (434)  GAGAGAAATATTCAAAGTGTTCGAGCTGAA--------------------
```

Figue 31 continued

```
                                    551                                              600
         AF187322 Canis IL-4  (493) TACATAGCTTTATTTTTAAAATATTTATA-ATTTATA------GATAATA
NM_000589 Homo sapien IL-4  (549) TG-ATAGGTTTATTTTTTAAGTATTTATATATTTATAACTCATCATAAAA
    U19838 Cercocebus IL-4  (464) --------------------------------------------------

                                    601                                 637
         AF187322 Canis IL-4  (536) TAA--TATATATAGACTCTAATATAAAAAAAAAAAAA
NM_000589 Homo sapien IL-4  (598) TAAAGTATATATAGAATCTAA----------------
    U19838 Cercocebus IL-4  (464) -------------------------------------
```

Figure 32

Evolution of polypeptides by synthesizing (in vivo or in vitro) corresponding deduced polynucleotides and subjecting the deduced polynucleotides to directed evolution and expression screening subsequently expressed polypeptides.

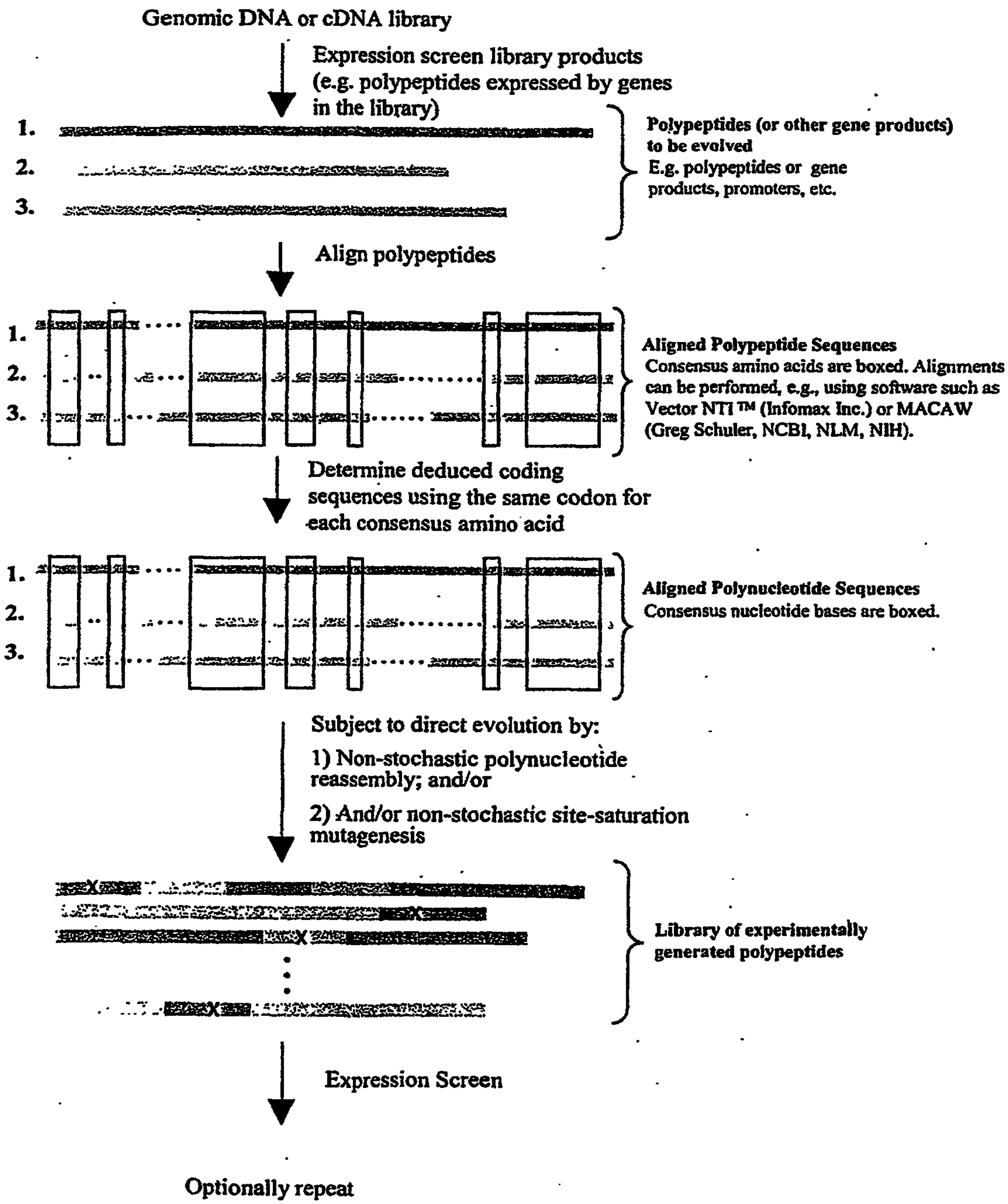


Figure 33

Directed evolution of polynucleotides (e.g. promoter sequences)

This figure shows an example of the application of non-stochastic site-saturation mutagenesis in combination with non-stochastic reassembly (e.g. oligo-directed CpG deletion(s) and/or addition(s))

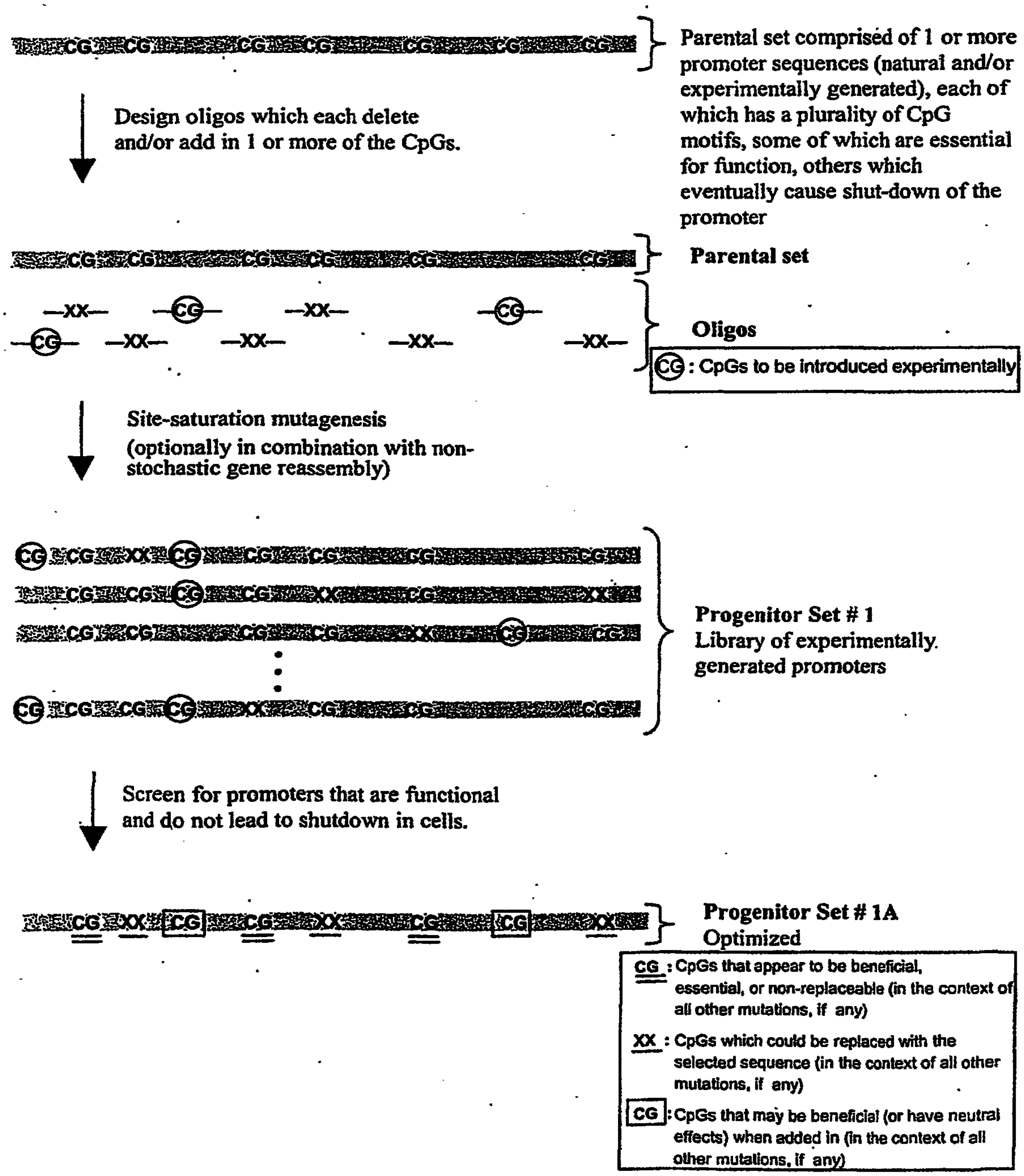


Figure 34

An example of a CTIS obtained from HbsAg polypeptide (PreS2 plus S regions).

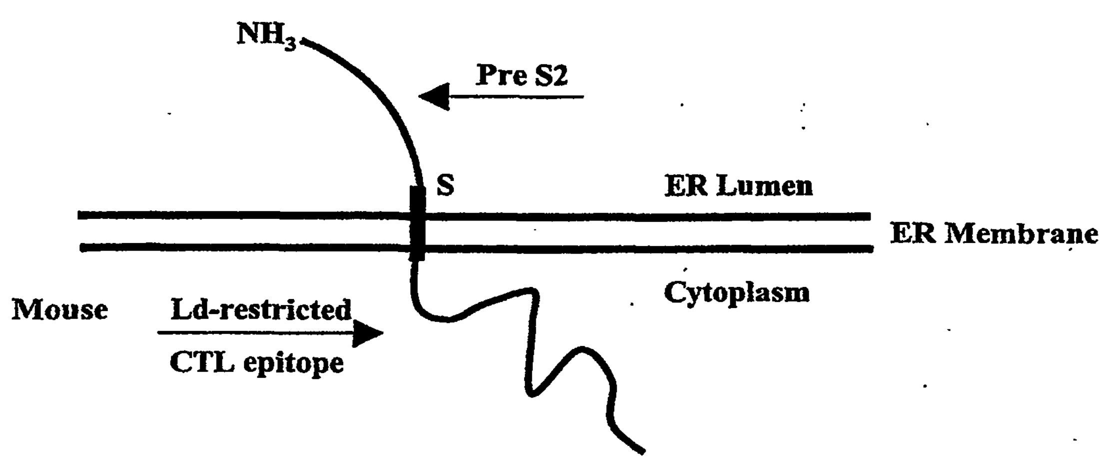


Figure 35

An example of a CTIS having heterologous epitopes attached to the cytoplasmic portion.

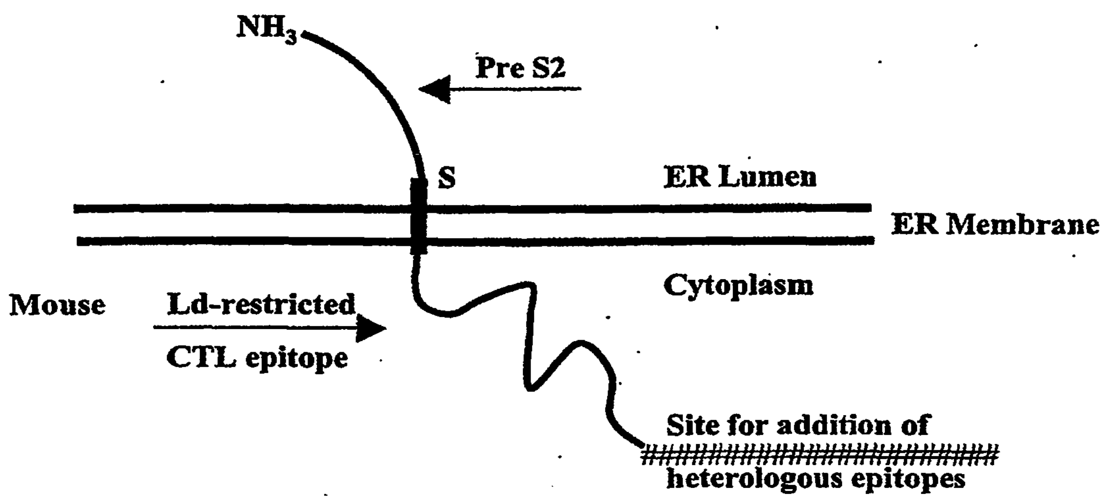


Figure 36

Method for preparing immunogenic agonist sequences (IAS).

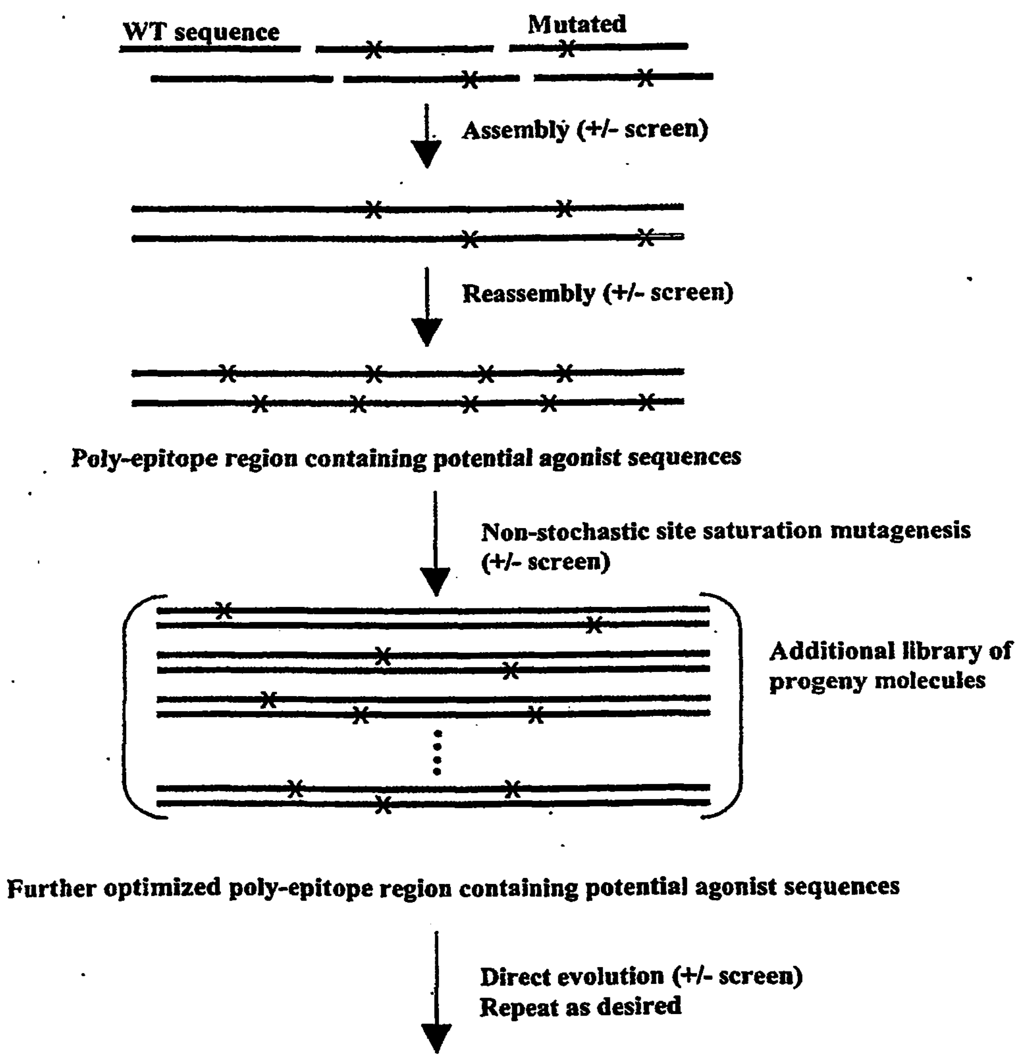


Figure 37

Improving Immunostimulatory Sequences (ISS) Using Directed Evolution.

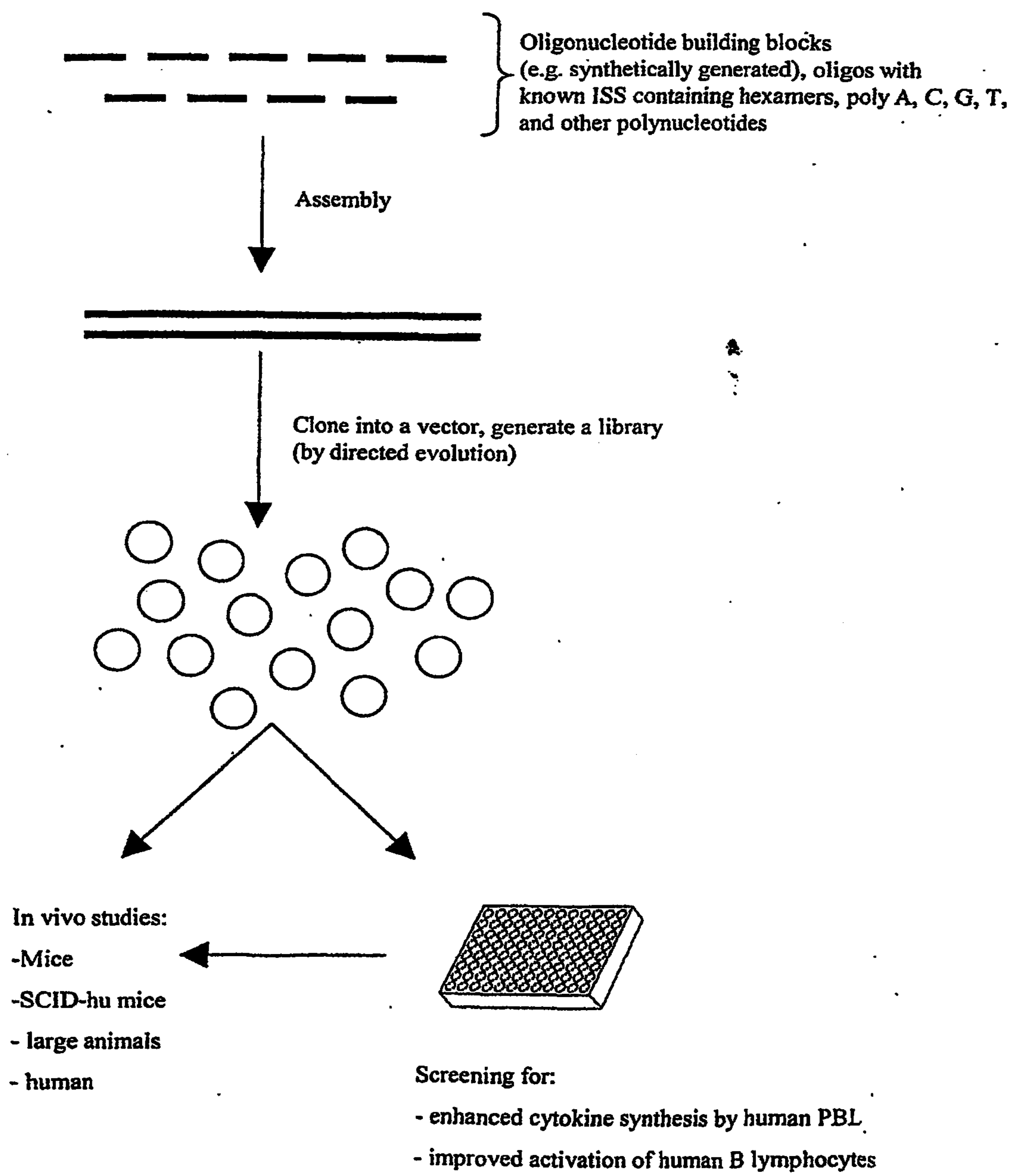


Figure 38

Screening to identify IL-12 genes that encode recombinant IL-12 having an increased ability to induce T Cell proliferation.

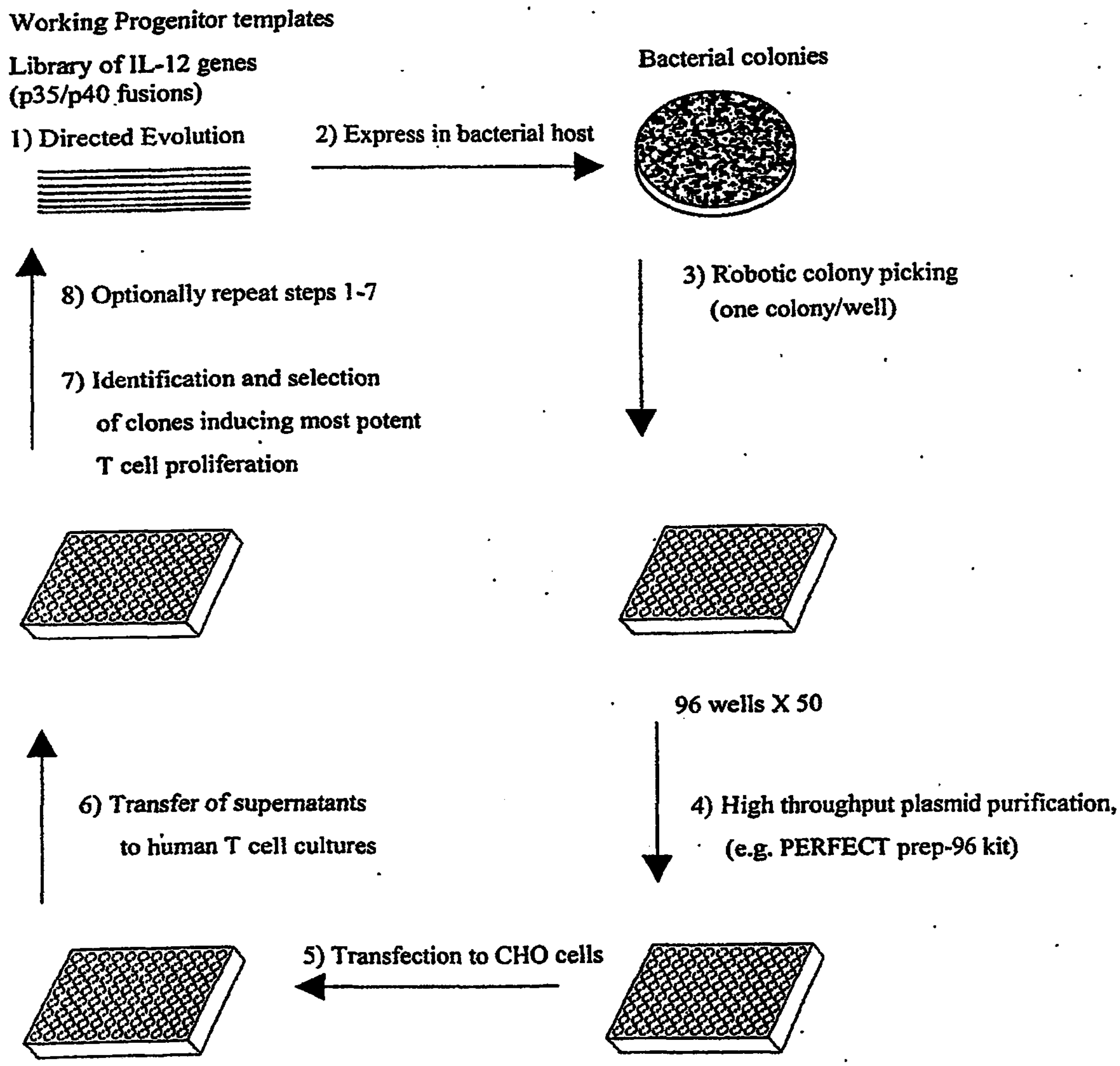


Figure 39

Model of induction of T cell activation or anergy by genetic vaccine vectors encoding different CD80 and/or CD86 variants.

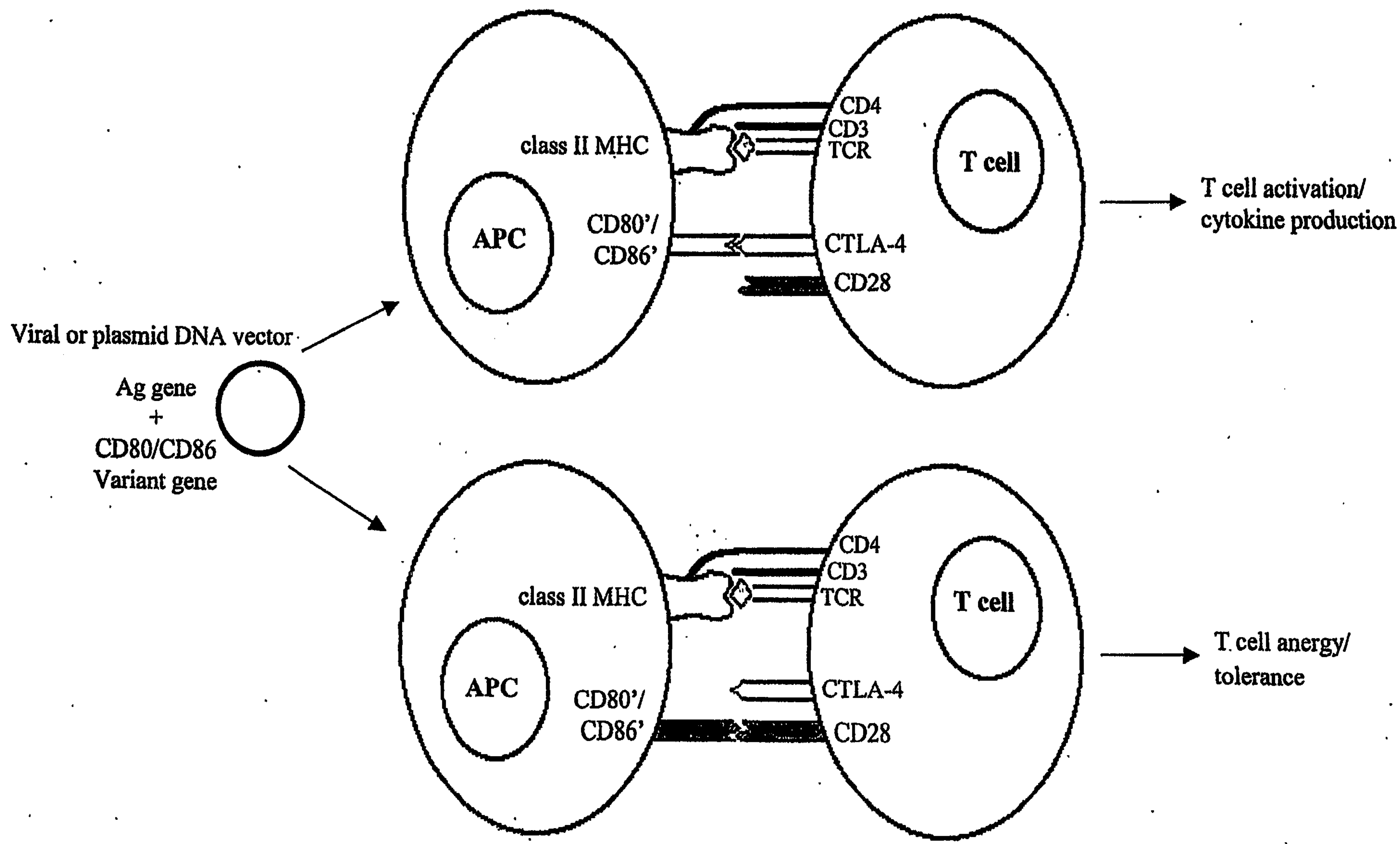


Figure 40

Screening to identify CD80/CD86 chimeric genes having an improved capacity to to induce T Cell activation or anergy.

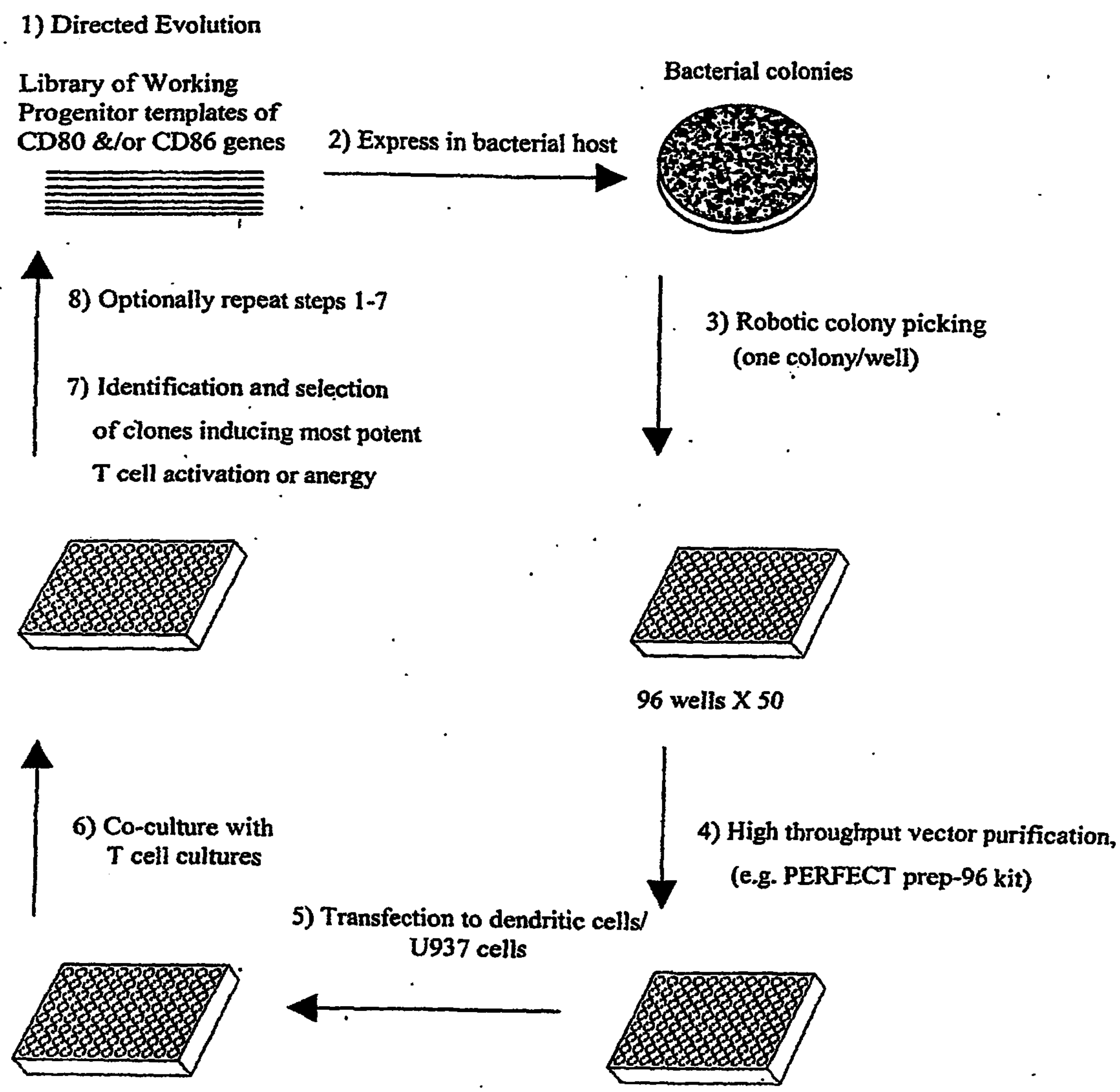


Figure 41

Figure 41. An alignment of two CMV-derived nucleotide sequences from human and primate species.

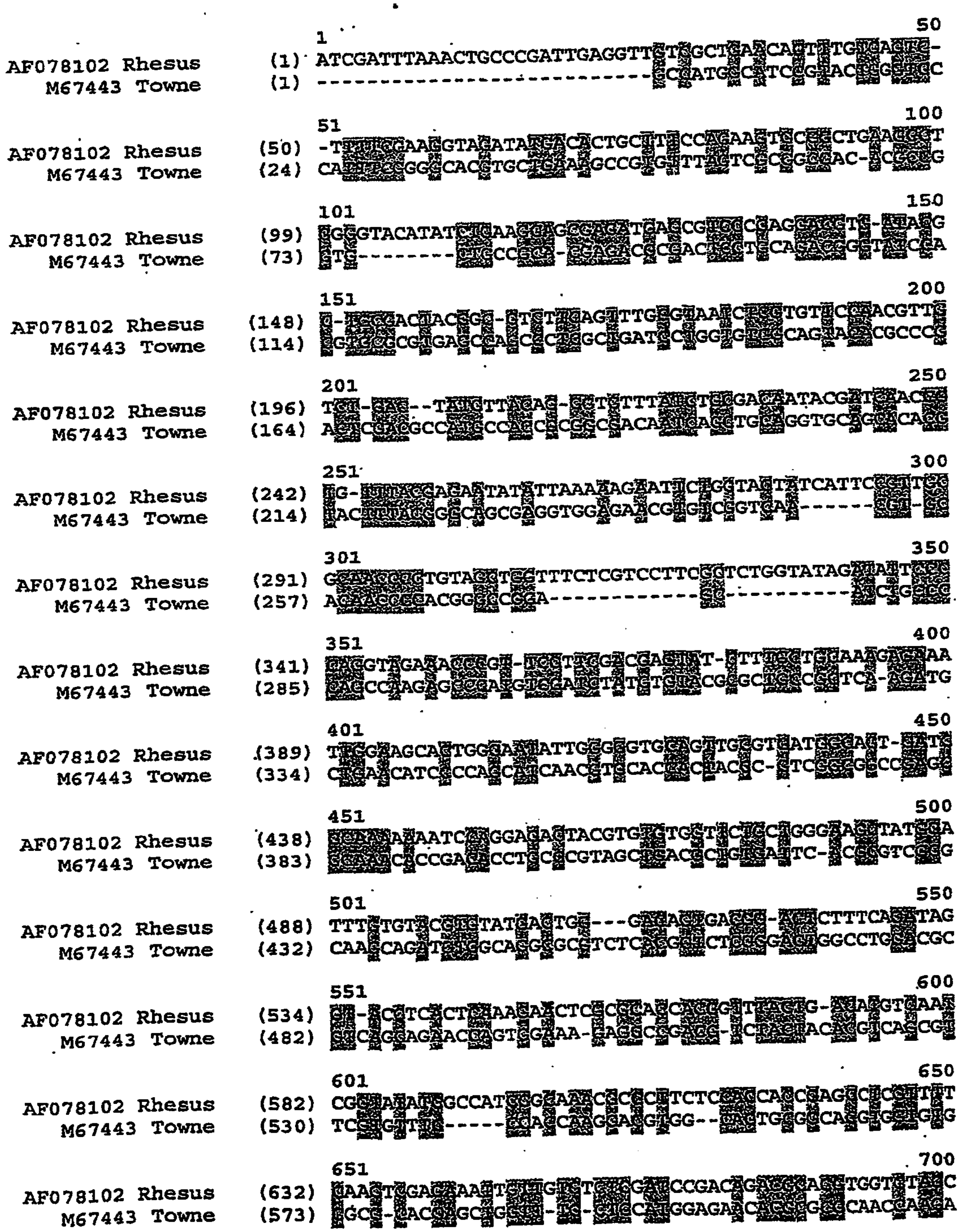


Figure 41 continued

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                          701                                              750
AF078102 Rhesus   (682)   TAAGACCGCAGAAGAGTTTCATGGCGTTAACGTAGTAGTGAAAAGGC-GT
   M67443 Towne   (620)   T--GCAGGTGATAGGTGACCAGTACCTCAAGGTGTACCTGGAGTGCTTGG

                          751                                              800
AF078102 Rhesus   (731)   GGCCGATCGGAGGCCGATCCGTTCCTGCTGCTGG--GTACTGTGGAGAAG
   M67443 Towne   (668)   GCGAGGACGT-GCGGTCCGGCAACGGTTTTATGCACGTCACGCTGGGCTC

                          801                                              850
AF078102 Rhesus   (779)   CTACGTCAAATGTATGGCTTTGCTAAAATGAGAGGTGATAAGTTTAGTAA
   M67443 Towne   (717)   TGACCTGGAAGAGGAGGTGACGATGACCCGCAACCGCAACCGCTCAGGC

                          851                                              900
AF078102 Rhesus   (829)   TGTG--ATAAAGTATATTTAATCAAGGCATGTCGTGCCGA------TGGT
   M67443 Towne   (767)   GGCGCCACGAGCGCAGCGGCTTTACGGTGTTGTGTCGCAAAAATATGATA

                          901                                              950
AF078102 Rhesus   (871)   --CGTACTTGGTGTTA-GTGGC-CGTTACCGCTAATATTGGAAATTTGCTG
   M67443 Towne   (817)   ATCAAAGCGGGCAAGATGTCGGACATCATGCTGGATGTCGCTGGTACGTC

                          951                                             1000
AF078102 Rhesus   (917)   AGCTGTGGAATAATTCTGTTGGACGAGTGCGGCGTGTGTTATGTGG-TGA
   M67443 Towne   (867)   ACACGAGCATTT--TTGGGCTGCT-GTGTCGCAAGAGCATCCCGGGGCTGA

                          1001                                            1050
AF078102 Rhesus   (966)   GAATGGACGAAAGCGACGTGTTT--CGTCTAGGCGATAAGATCCAAATGT
   M67443 Towne   (914)   GCATGTCAGGTAAGCTATTGATGAACGGGCAGCAGAT--GTTCGTGGAGG

                          1051                                            1100
AF078102 Rhesus  (1014)   TGTTCAGATGCCGGCTTTGTTAAGCTCCGTGGCATTACGCGATTTGAGCGC
   M67443 Towne   (962)   TGC----AAGCGA--TAGGCGAGA-CCGTGCAACTGCGTCAGT--AG---

                          1101                                            1150
AF078102 Rhesus  (1064)   GGATTGCCTGCCGAGGCTCGTCTG----GAGAGTGAGT-GCTACTGGTAT
   M67443 Towne  (1000)   -GATCCCGTGCCTGCGCTGTTCGTTTTCGATATCGACTTGCCGCTGG-AG

                          1151                                            1200
AF078102 Rhesus  (1109)   GAGGGTGGCCGGTAAGCAAGATGTGTGTTGGTGCTGTATGGTCAACACAGT
   M67443 Towne  (1048)   CGGCGGCCGTGAGTACAGCGAAGAGCGCACCCTTGAGCA--GCGAGTATCGC

                          1201                                            1250
AF078102 Rhesus  (1159)   TAGCGCCGAGGAGCTCTGTGGTGCGT-AGGATTGGCTCAGGCGGAGTAAT
   M67443 Towne  (1096)   ATCGA--GGCGAAGCTTGAGTACCGACACACACGTGCG--AGCGGCACGACG

                          1251                                            1300
AF078102 Rhesus  (1208)   CGCGCTGATGATAAAATACGTAGCAGTTGGTTGTTGGCGACGTAGTGATTT
   M67443 Towne  (1142)   AGGGTGGCGCCCAGGGCGACGACGACGTCTGGACCAGCGGATCGGACGCC

                          1301                                            1350
AF078102 Rhesus  (1258)   GATGCCTACCGGAGTTATTGCAACAGAATCAGAATTGGTGCTTTCGGTGGA
   M67443 Towne  (1192)   GACGAGGAAGTC-GTAACCACCGAGCGCAAGACGCCCCGCGTTAGCGGCG

                          1351                                            1400
AF078102 Rhesus  (1308)   GGAGTGTGGCGGTTCTAGAAGGGCG-GCAAAGCGGTGCGTGGGAG--GAG
   M67443 Towne  (1241)   GCGGCGCGGATGG-----GGGGCGGGTCGACTTGCGCGGGCCGGAAACGCA
```

Figure 41 continued

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                                1401                                              1450
AF078102 Rhesus      (1355) AATGATGAGTTAACGGAGAAAACGTCTGATGGTACTGTGATGTTTGGTGT
  M67443 Towne       (1286) AATCAGCA-TCCTCGGCGACGGCCTGCACGGCGGGCGTTATGACACGCGG

                                1451                                              1500
AF078102 Rhesus      (1405) A-GACAGAGGTTTTTCTCCCAGAAGCGTAAAGTTGGAGGGTGAAAGCCAT
  M67443 Towne       (1335) CCGCCTTAAGGCCGAGTCC----ACGCTCGCGCCGGAAGAGGACACCGAC

                                1501                                              1550
AF078102 Rhesus      (1454) GACTACAGAGAAGAGGAGGAAGATGATGATAACCAGTCAGAAGAAACCCC
  M67443 Towne       (1381) GAGGATTGCGACAACGA--AATCC----ACAATGCGGCCGTGTTCAGCTG

                                1551                                              1600
AF078102 Rhesus      (1504) TCAGGATCAGCCTGTTGTCATTGTTTGAGATGAAACAAACGGCAGTGTTG
  M67443 Towne       (1425) GGCGGCCTGGCAGGCCGGCATCCTGGGCAGCAACCTGGTGCCCATGGTTG

                                1601                                              1650
AF078102 Rhesus      (1554) ATTGTTATGATGATGAGAGTGAGTCAT-GTCTG--TCGT-CAGATAGCCA
  M67443 Towne       (1475) CTACGGTTCAGGGTCAGAATCTGAAGTAGCAGGAGTTCTTGTGGAGGGC

                                1651                                              1700
AF078102 Rhesus      (1600) TGATGCAGCAGCCCCAGAGCATTATGGCCATAATCCAAAAGGAATCGCA--
  M67443 Towne       (1525) --AACGACATGTACGG--CATCTTCGCCGAATTGGAAGGCGTATCGCAGC

                                1701                                              1750
AF078102 Rhesus      (1648) ----------AAACGAAGAGCGGGTTAGCAATGAAAGAGAATTGTTAGTGC
  M67443 Towne       (1571) CCGCTGCGCAACGCAAACGTCGCCGCGACCGGCAAGACGCCT-TGCGCGG

                                1751                                              1800
AF078102 Rhesus      (1689) GGCGTGCA---CGACGA-GGTAAACATATGAAAAAGTGGCATTGTTGCCT
  M67443 Towne       (1620) GCCATGCATCGGCTCGACGGCCAAAAAGCACCGAGGTTGAGCCACCGGCC

                                1801                                              1850
AF078102 Rhesus      (1735) GGAAAGGGTTAGCA------TTTATGACCCGTTGAATGCTAATATTGAGT
  M67443 Towne       (1670) GCGCACGCTTAGGACGACTCTATAAAAACGCACGTCCAGTCAGACAGGCG

                                1851                                              1900
AF078102 Rhesus      (1779) GCTTGATTAAACAATGCATTTGTATTGAATTATCTAATTCGGGT--GTA
  M67443 Towne       (1720) AGTTTGGCCGCCGA---CACCTGTCGCCGCTGCTATATTTGGCACAGTTG

                                1901                                              1950
AF078102 Rhesus      (1827) CCGTGTCATTTGTTCCATGCAGTGGTGCGAAAGAGATTAGCTATTTAGACG
  M67443 Towne       (1767) CCGGAAGCCTTCCGGA--GCTCCCACGAAGAACGGTT-CACCTTGCCGCG

                                1951                                              2000
AF078102 Rhesus      (1877) -CACGTATGAGTTGTGAAAGCTGAGTCAACATCATGCAATTGTGCGACGA
  M67443 Towne       (1814) TGCCCTGAGCGCCCCC----GTCATCGCGCCTCGCGATGTCCTCAGGCAT

                                2001                                              2050
AF078102 Rhesus      (1926) GCTCCGGGTCGTTCGTTCTGTGTTTGTT-GTTACGAGTTTAGATTTTATTA
  M67443 Towne       (1860) CGTCG---TCGCCCGGTGAGGCACCGTCGTCGGAAGCGGCGGCGATCAGC

                                2051                       2077
AF078102 Rhesus      (1975) TGGTTGAAAGTGTTGTATTTCTGA---
  M67443 Towne       (1907) GAGGCGGAGCCGGCG-AGCGGAAGCTT
```